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UTILIZATION OF FRUCTOSE BY THE NERVOUS SYSTEM IN MAN*

R. F. TAGNON, M.D. AND J. CORVILAIN, M.D.
Laboratory of Experimental Medicine and Department of Internal Medicine, University of Brussels, Brussels, Belgium

ABSTRACT

In 3 groups totaling 38 subjects, concurrent administration of fructose with insulin was shown to prevent the clinical symptoms of hypoglycemia, hypoglycemia-induced eosinopenia and hypoglycemia-induced electroencephalographic changes without increasing the level of venous blood glucose, as compared to control studies in which insulin alone was injected. In contrast, fructose did not prevent the effect of hypoglycemia in increasing gastric secretion of hydrochloric acid. These results seem to demonstrate that at least some parts of the brain are able to utilize fructose or metabolites of fructose.

THE metabolism of fructose by the normal and the diabetic human organism has been a subject of investigation for many years. It is generally recognized that the main site of fructose metabolism is the liver and, to a lesser degree, the kidney and intestinal mucosa. It is likely that other tissues such as muscle and adipose tissue may also use fructose under certain circumstances, although not in the presence of significant quantities of glucose.

The utilization of fructose by nervous tissue has been negated on at least two grounds: 1) The relative lack of permeability of nervous tissue to fructose, and 2) the absence of the specific enzyme, fructokinase, in this tissue.

The present study was performed in order to determine whether clinical

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evidence could be gathered as to whether or not nervous tissue is capable of utilizing fructose at low concentrations of blood glucose. To this end, the effect of administration of fructose upon some of the signs and symptoms of insulin hypoglycemia was investigated.

MATERIALS AND METHODS

None of the 38 subjects exhibited any evidence of anomalies of the nervous system or carbohydrate metabolism. In all instances the experimental observations were divided into 2 parts. In the first part, each subject received 0.1 unit of crystalline insulin per Kg. of body weight, injected intravenously, and venous blood samples were collected at five-minute intervals for one hour. In the second part (usually performed two days later), in addition to the insulin, an intravenous infusion of 10 per cent fructose in water was initiated and continued for one hour. The total amount of fructose infused into each subject was 90 Gm. Again, venous blood samples were collected at five-minute intervals throughout the hour. In each case the blood samples were analyzed for total reducing substances by the method of Shaffer and Hartman (1), for fructose by the method of Roe *et al.* (2), and for glucose by subtraction of the fructose value from that for total reducing sugar.

Insulin hypoglycemia is characterized by a number of signs and symptoms, among which we emphasize 1) a clinical syndrome including sweating, tremor, tachycardia and the sensation of hunger; 2) alterations of the electroencephalographic tracing; 3) decreased levels of circulating eosinophilic leukocytes (presumably due to activation of the pituitary-adrenal system); and 4) increased gastric secretion of hydrochloric acid (presumably due to stimulation of hypothalamic centers and vagal nerves). Simultaneous studies of all of these indices in each subject did not prove practical. Accordingly, the 38 subjects studied were subdivided into 3 groups. In all, the clinical syndrome and the influence of fructose thereon was noted.

In 14 patients, eosinophil counts were performed both before, and four hours after the injection of insulin, in both parts of the study.

In 12 patients, electroencephalographic tracings were continuously recorded from ten minutes prior to injection of insulin until one hour after the injection. Whenever a significant hypoglycemic alteration was noted during the first part of the study, *i.e.*, when insulin alone was injected, a similar electroencephalographic tracing was obtained during the second part of the study, *i.e.*, when fructose was injected in addition to insulin. The complete set of observations was obtained in 4 patients. In 1 of them (C.A.), the insulin dose was doubled when insulin and fructose were administered together.

In 12 patients, the gastric secretion of hydrochloric acid was measured.

RESULTS

Clinical syndrome

Clinical symptoms typical of hypoglycemia were observed in 12 of the 38 patients after the injection of insulin alone, but never when fructose was administered simultaneously, although no significant difference in the degree of the hypoglycemia could be observed. In Table 1 is a summary of the clinical status of the 12 subjects when the concentration of blood glucose had reached its lowest level following injection of insulin, with or without fructose.

TABLE 1. CLINICAL SYMPTOMS OF HYPOGLYCEMIA IN 12 SUBJECTS, MANIFESTED AT THE LOWER BLOOD GLUCOSE LEVEL REACHED AFTER: 1) INSULIN, 2) INSULIN PLUS FRUCTOSE

Patient	Dose of I.V. insulin	Lowest blood sugar level (mg./100 ml.)	Sweating	Malaise	Confusion
C.D.	7U. 7U.+Fructose	34 14	+ 0	+ 0	0 0
R.A.	5U. 5U.+Fructose	7 8	++ 0	++ 0	+ 0
L.G.	6U. 6U.+Fructose	25 9	++ 0	+ 0	0 0
V.G.	7U. 7U.+Fructose	6 0	++ 0	+ 0	+ 0
L.B.	8U. 8U.+Fructose	5 10	++ 0	++ 0	+ 0
J.F.	6U. 6U.+Fructose	6 15	+++ 0	+++ 0	++ 0
A.V.	7U. 7U.+Fructose	15 22	+++ 0	++ 0	++ 0
C.A.	7U. 14U.+Fructose	5 0	+++ 0	++ 0	+ 0
P.V.	7U. 7U.+Fructose	21 11	++ 0	+ 0	+ 0
C.J.	6U. 6U.+Fructose	9 20	+ 0	+ 0	0 0
J.P.	7U. 7U.+Fructose	2 2	++ 0	++ 0	+ 0
F.R.	6U. 6U.+Fructose	5 4	+ 0	+ 0	0 0
Mean value	*Insulin* *Insulin+Fructose*	*11.6* *9.6*			

Circulating eosinophils

In 14 subjects, the injection of insulin alone depressed blood glucose levels to a mean value of 24 mg. per 100 ml. at twenty-five minutes (Fig. 1). When insulin and fructose were administered concurrently the hypoglycemia produced was virtually the same—a mean value of 18 mg. per 100 ml. at twenty-five minutes. On the other hand, the concentration of total reducing sugar remained at levels around 90–100 mg. per 100 ml. during administration of fructose.

In contrast (Fig. 2), the marked eosinopenia produced after the injection of insulin alone was not present when insulin and fructose were administered together.

Electroencephalogram (EEG)

Of 12 subjects tested, only 4 showed definite changes in the EEG after

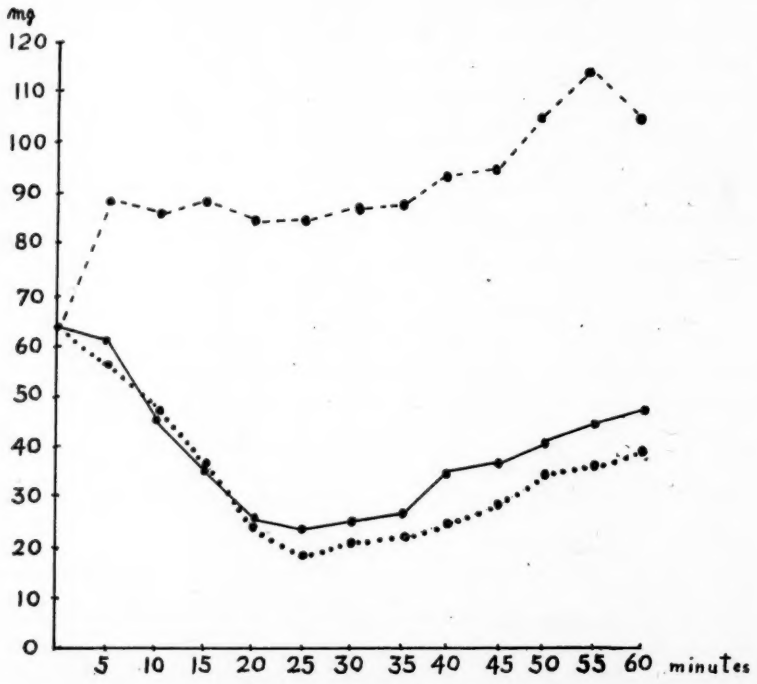

FIG. 1. Mean venous blood levels of glucose and total reducing sugar (mg./100 ml.) in 14 subjects. The solid line represents blood glucose values after injection of insulin (0.1 unit/Kg.) during the first part of the study. The dotted line represents blood glucose values after injection of insulin (0.1 unit/Kg.) and fructose (90 Gm. of a 10% solution in one hour) during the second part of the study. The broken line at the top of the graph represents the total reducing sugar values during the second part of the study.

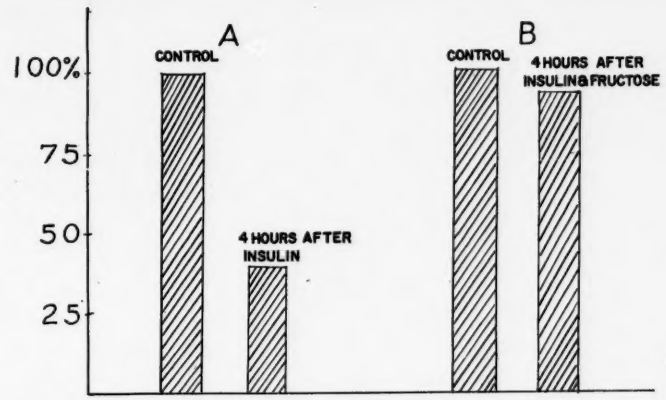

FIG. 2. Decrease of the blood eosinophil level, expressed as a percentage of the initial value. **A.** Four hours after injection of insulin (0.1 unit/Kg.). **B.** Four hours after injection of insulin (0.1 unit/Kg.) and fructose (90 Gm. of a 10% solution in one hour). (Mean values in 14 subjects.)

administration of insulin alone (0.1 unit per Kg.). In these 4 the blood glucose levels were depressed to 21 mg., 0 mg., 6 mg. and 5 mg. per 100 ml., respectively. The EEG abnormalities, which consisted in the appearance of slow waves and decreased amplitude of the complexes, were noted twenty minutes after injection of insulin. In the 4 cases, these abnormalities were completely absent in the second part of the study when fructose was injected together with insulin. The blood sugar levels reached during this period were (listed in the same order as before) 11 mg., 0 mg., 15 mg. and 0 mg. per 100 ml. The values for reducing sugar, however, were maintained above the initial glucose levels throughout.

These findings were particularly clear-cut in Subject C.A. whose EEG was markedly altered twenty-five minutes after intravenous injection of 7 units of insulin (Fig. 3, A and B). On the contrary, following simultaneous injection into the same subject of twice that dose of insulin (*e.g.*, 14 units) along with fructose, there was no change in the EEG tracing (Fig. 3, C and D) although the blood glucose level remained at about zero for twenty minutes (Fig. 4).

Gastric secretion of hydrochloric acid

In these 12 subjects the injection of insulin alone decreased the mean blood glucose level to 19 mg. per 100 ml., whereas the administration of insulin and fructose together resulted in a mean level of 11 mg. per 100 ml., despite total reducing sugar values above initial glucose values.

As shown in Figure 5, a marked increase in free gastric hydrochloric acid occurred, both after insulin alone and after insulin and fructose. The increases were about the same in both cases.

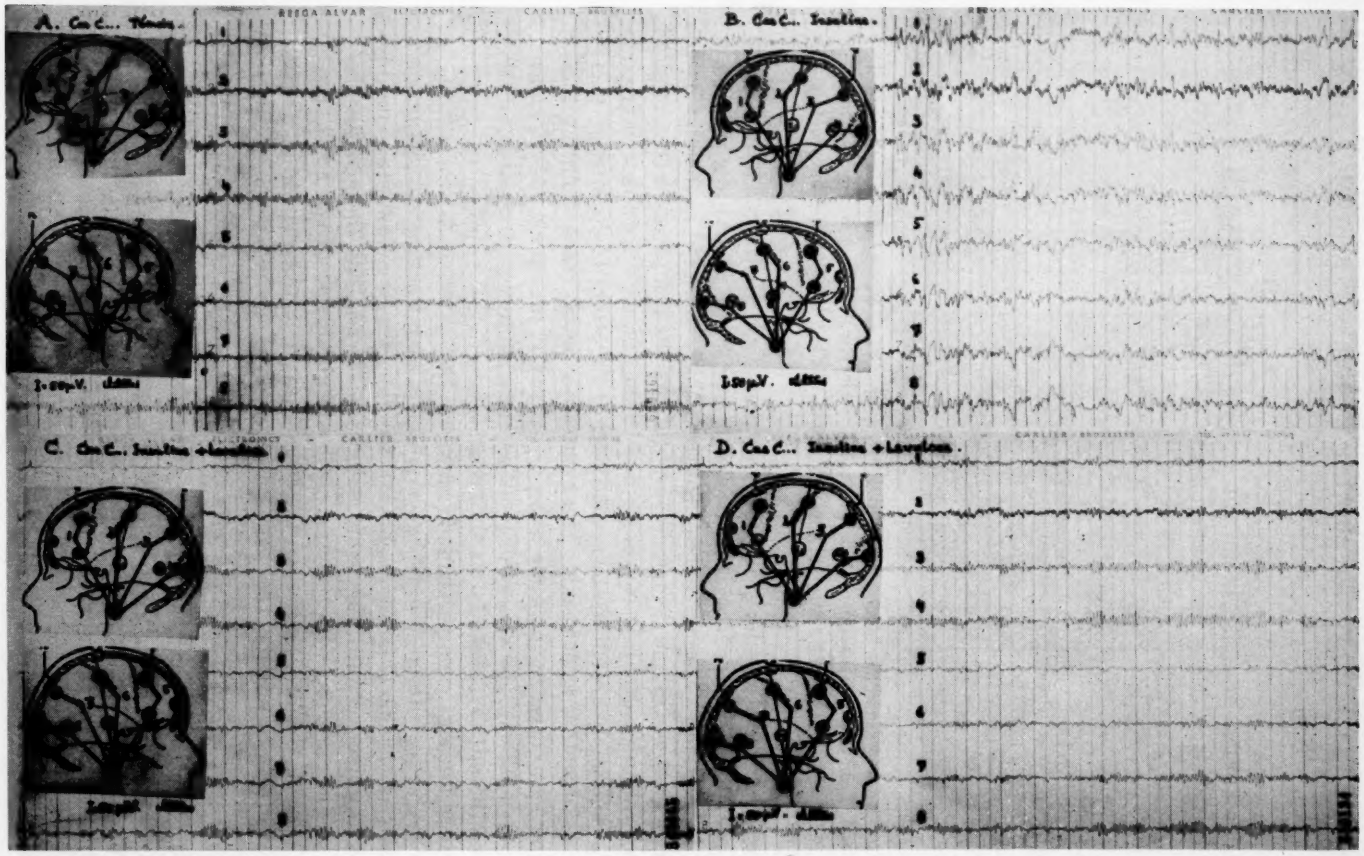

FIG. 3. Electroencephalograms, *Patient C.A.* **A.** Control tracing before injection of insulin. **B.** Tracing 25 minutes after injection of insulin; appearance of slow waves. **C.** Tracing 25 minutes after injection of insulin and fructose; normal as compared to control. **D.** Tracing 45 minutes after injection of insulin and fructose; normal as compared to control.

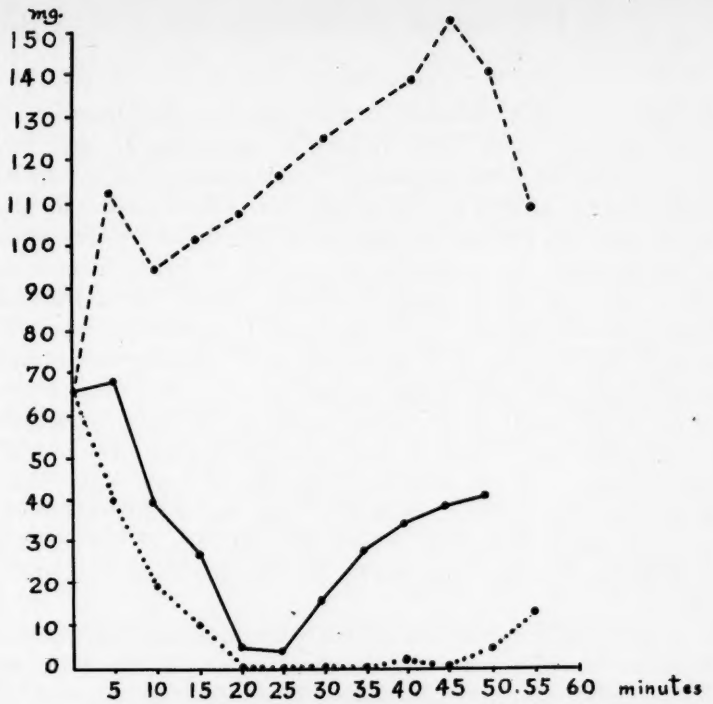

FIG. 4. Mean venous blood levels of glucose and total reducing sugar (mg./100 ml.) in *Patient C.A.* The solid line represents blood glucose values after injection of insulin (0.1 unit/Kg.) during the first part of the study. The dotted line represents blood glucose values after injection of insulin (0.2 unit/Kg.) and fructose (90 Gm. of a 10% solution in one hour) during the second part of the study. The broken line at the top of the graph represents the total reducing sugar values during the second part of the study.

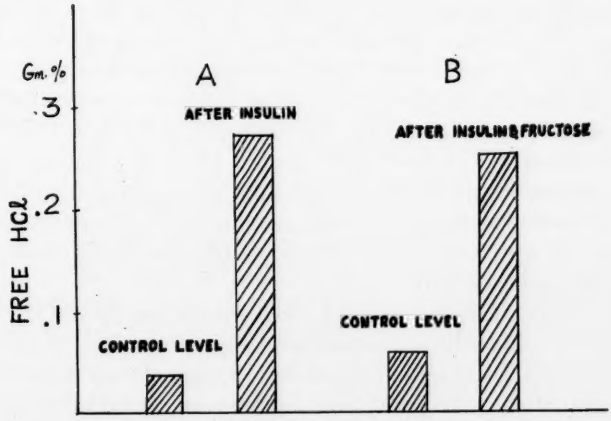

FIG. 5. Mean levels of gastric hydrochloric acid in 12 subjects. **A.** Increase in gastric free HCl after injection of insulin (0.1 unit/Kg.). **B.** Increase in gastric free HCl after injection of insulin (0.1 unit/Kg.) and fructose (90 Gm. of a 10% solution in one hour).

DISCUSSION

It has been generally assumed in the literature that fructose is not utilized by nervous tissue (3–6). Indeed, a mechanism for this lack of fructose utilization has been suggested by enzymatic studies *in vitro* which have shown that the affinity of brain hexokinase is much greater for glucose than for fructose and that in the presence of equimolar concentrations of glucose and fructose, the utilization of fructose by brain tissue is nil or minimal. In the absence of glucose, however, utilization of fructose by brain tissue proceeds rapidly (7). On the basis of this mechanism alone one might expect therefore that *in the presence of severe hypoglycemia* utilization of fructose by nervous tissue might proceed at a rate sufficient to prevent some or all of the metabolic anomalies which accompany hypoglycemia. However, Bollman and Mann (3) have reported the inability of fructose to relieve hypoglycemic convulsions in eviscerated dogs, suggesting that nervous tissue cannot utilize fructose even when marked hypoglycemia has occurred. This observation is in accord with the lack of permeability of nervous tissue for fructose suggested by the experiments of Park and associates (4).

In the present study carried out in man it would appear that the concurrent administration of fructose with insulin can prevent the clinical symptoms of hypoglycemia, hypoglycemia-induced eosinopenia, and hypoglycemia-induced electroencephalographic changes. EEG changes are infrequent in short-term hypoglycemia and appeared significantly only in 4 of the 12 subjects tested. It is possible that an EEG tracing which showed alterations during one period of insulin-induced hypoglycemia might not show the same abnormalities during a second experimental period. In that case, the alleviation of EEG abnormalities after administration of fructose would appear to be purely fortuitous. This interpretation, however, seems unlikely, since all 4 subjects in whom definite alterations of the EEG tracing were noted during insulin-induced hypoglycemia had a completely normal EEG during insulin-induced hypoglycemia accompanied by significant fructosemia. Furthermore, in Subject C.A. there was marked alteration of the EEG with insulin alone (0.1 unit per Kg.), but no alteration was seen with twice that dose given along with fructose. As the latter produced prolonged hypoglycemia (Fig. 4), it appears reasonable to attribute the absence of EEG abnormalities during the second trial to the administration of fructose.

Since all of the consequences of hypoglycemia are presently thought to be mediated by metabolic abnormalities of nervous tissue, either generally or in critical areas, these observations would suggest that either fructose or fructose metabolites may be utilized by nervous tissue in the presence of grossly lowered concentrations of blood glucose.

Of possible fructose metabolites, glucose might be provided by gluconeogenesis in the liver in such an amount as to alleviate the symptoms of hypoglycemia. The fact that administration of fructose did not reduce the degree of hypoglycemia provoked by insulin as compared to periods when insulin alone was injected, is not in favor of this hypothesis, but it cannot be rigorously ruled out without arterial blood glucose determinations.

Our results point rather to utilization by the nervous system of fructose itself, or of a metabolite of fructose other than glucose. However, the work of Seltzer *et al.* (8) published recently in abstract seems to contradict our conclusion. These authors failed to demonstrate any cerebral arteriovenous difference of fructose, lactate or pyruvate levels during injection of insulin plus fructose in an amount sufficient to prevent clinical symptoms.

On the other hand, fructose did not prevent the effect of hypoglycemia upon gastric secretion of hydrochloric acid, since the difference between the levels of gastric hydrochloric acid reached after injection of insulin alone and after injection of insulin plus fructose was not significant. The reason for this discrepancy is not clear at the present time, but it suggests the presence of metabolic differences between separate areas of the nervous system.

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ASSAY OF HUMAN PITUITARY GONADOTROPIN FROM MALE AND POSTMENOPAUSAL URINE

A. ALBERT, M.D.* AND E. ROSEMBERG, M.D.†

Mayo Clinic and Mayo Foundation, Rochester, Minnesota; the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; and the Dementia Praecox Research Unit Project, Worcester State Hospital, Worcester, Massachusetts

ABSTRACT

Human pituitary gonadotropin (HPG) was prepared from male urine and from postmenopausal urine by the same process and was assayed by four methods, namely by ovarian weight in intact rats, by uterine weight in intact mice, and by the ventral-prostatic weight and testicular weight in hypophysectomized rats. The relative potency (postmenopausal ÷ male) of the two preparations of HPG was 12.83 by the four methods of assay (total of 14 assays). When the specific activities of the two preparations of HPG were equalized by appropriate dilution of the more potent HPG (postmenopausal) with inert material, the relative potency was 1.12 by the four methods of assay (total of 14 assays). The mean index of discrimination using all possible combinations of assays was close to unity, and all values for the mean index of discrimination fell well within experimentally verified and allowable ($P = 0.95$) limits of fluctuation. It was concluded that no evidence for dissimilarity of HPG from these two sources was obtained by these conventional methods of assay.

TWO previous collaborative assays of human pituitary gonadotropin (HPG) from male and from menopausal-postmenopausal or castrated women have been reported by Albert and associates (1, 2). It was observed that HPG from men and from postmenopausal or castrated women could be assayed in terms of each other by two widely used methods, namely ovarian weight in rats and uterine weight in mice. However, variations in assay results were noted. These variations could have arisen from differences in the method of preparation of HPG, in the amount of inert material present in various preparations of HPG and in the proportions of active hormonal materials, and from interassay errors.

The present study was undertaken to reduce these sources of variation. The same general types of assays were used, but with the following restric-

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tions: 1) HPG was prepared from male and from postmenopausal urine by exactly the same chemical process, thus removing variation due to difference in methodology, and 2) variation arising from differences in content of inert material was minimized by diluting the more potent preparation of HPG with inert material so as to equalize the amount of inert material.

MATERIAL AND METHODS

Urine was obtained from normal men and was pooled as described previously (3). The urine was processed in batches of 6 liters each by the routine kaolin-acetone method (4, 5). Eighteen of such 6-liter batches were processed, the extracts being dried and thoroughly mixed. The yield of solid material per batch varied from 37 to 74 mg. per liter (mean = 52 mg.). The total yield of material from the 108 liters was 5.6 Gm. This preparation will be referred to as "preparation A." A healthy postmenopausal woman contributed complete 24-hour specimens of urine for eleven consecutive days (6). The 24-hour specimens of urine were processed in identical fashion. The yield of solids varied between 17 and 36 mg. per liter of urine (mean = 25 mg.). The total material from the 22.43 liters of urine comprising the eleven-day collection was 522 mg. This material will be referred to as "preparation B."

Preliminary assay of preparation A by the ovarian-weight method (7) was carried out in 35 rats by means of one four-point assay and one three-point assay. Using the fixed-slope method, the mean potency of A was 0.31 rat unit per milligram. Because of the small amount of preparation B, only one three-point assay was performed on 15 rats. The mean potency of preparation B was 3.8 rat units per milligram. Thus, preparation B was 12.3 times stronger than preparation A. This preliminary estimation of relative potency was used as a guide for carrying out more precise tests.

Simultaneous assays of B in terms of A were carried out by four methods, namely by ovarian weight in intact rats, by uterine weight in intact mice, and by the ventral-prostatic weight and testicular weight in hypophysectomized rats. A two-and-two design was employed; two doses of A separated by a log interval of 0.301 and two doses of B separated by the same log interval were used in all assays. Doses of 20 and 40 mg. per animal of A, respectively, and doses of 1.5 and 3.0 mg. per animal of B, respectively, were used in the ovarian-weight method in rats (7). Each dose was administered to 5 rats, making a total of 20 rats per assay. In the uterine-weight method in mice (8), the two doses of A were 1.9 and 3.8 mg. per animal, respectively, and the two doses of B were 0.15 and 0.30 mg. per animal, respectively. These doses were administered to 10 mice, making a total of 40 animals per assay. In the assays using the weight of the ventral prostate and the weight of the testes in hypophysectomized rats, the doses of A were 10 and 20 mg., respectively, and the doses of B were 0.79 and 1.58 mg., respectively, per animal. These doses were administered to 5 animals, making a total of 20 animals per assay.

The relative sensitivity of the four end points used in this study is given in Table 1. These values were obtained in the following manner: The mean weight of the organs at the two doses of HPG was plotted on semilog paper. The dose intercept in milligrams was determined for the responses listed in Table 1. For example, the dose in milligrams of A that produced a 50 per cent increase in ovarian weight in rats was determined for each of the

assays of A. The mean of these values is given in Table 1. The same procedure was used to find the mean dose of A that doubled the weight of the uterus, prostate and testis. The entire procedure was repeated for B. Assigning a value of 1 for the sensitivity of the ovarian-weight method, the sensitivities of the other three are given by dividing their mean doses into the mean dose of A or B. The mean sensitivity shown in Table 1 is the average of the sensitivities determined for A and B.

In order to determine whether the absolute difference in the amount of inert material influences the assay, an inert fraction prepared from male urine was added to B so that the same amount of inert material was present in A and B. A total of 5 Gm. of material representing an inert, alcohol-insoluble fraction prepared from male urine (9) was dissolved in 500 ml. of 0.1 *M* borate buffer; 50 Gm. of sodium chloride was added and a suffi-

TABLE 1. SENSITIVITY OF END POINTS IN ASSAYING HPG

End point	Dose of preparation (mg.)		Mean sensitivity
	A	B	
50% increase in ovarian weight, rat	20.50	1.50	1
100% increase in uterine weight, mouse	2.70	0.26	7
100% increase in ventral-prostatic weight, rat	10.10	0.85	2
100% increase in testicular weight, rat	7.70	0.55	3

cient amount of a 1 per cent solution of sodium hydroxide was added dropwise to make the pH of the solution 9.0. The solution was boiled briskly for one hour in air. After the solution was cooled, glacial acetic acid was added dropwise until the pH of the solution was 5.5. Acetone (2 volumes) was added, and the resulting precipitate was removed by centrifugation and dried with alcohol and ether. No gonadotropic activity could be detected in this preparation at doses of 40 mg. per rat and 2 mg. per mouse. This is not proof of complete inactivity; however, the material was presumed to be inert for the purpose of the dilution experiment. An aliquot of preparation B then was diluted with this inert material in the proportion of 1 part of B and 11.6 parts of inert material. This ratio was chosen because the mean relative potency (B÷A) as determined by the ovarian-weight method in the rat and the uterine-weight method in the mouse was 12.6 (Table 2). The actual mixing was accomplished by placing 1 part by weight of B and 11.6 parts by weight of inert material in a 250-ml. Erlenmeyer flask, which then was secured to a shaking machine and agitated for two hours. The mixture then was ground in a mortar by hand and shaken for two more hours. This preparation will be referred to as "B (dil.)." It

was assayed simultaneously with A, using the same four end points. In all these assays, the two doses of A separated by a log interval of 0.301 were equal to the two doses of B (dil.).

The preparation of A, B and B (dil.) and the assays using the ovarian-weight method in rats were carried out in Rochester. Assays using the uterine-weight method, and the ventral-prostatic and testicular-weight methods in hypophysectomized rats were carried out in Worcester.

TABLE 2. ASSAY OF POSTMENOPAUSAL HPG (B) IN TERMS OF MALE HPG (A)*

End point	No.	N.	λ	R.P.	Confidence limits†
Ovarian weight, intact rats	1	20	0.04	12.66	(11.44–13.87)
	2	20	0.08	12.82	(10.38–15.29)
	3	20	0.07	10.99	(9.27–12.89)
	4	20	0.07	13.89	(11.89–16.17)
	5	20	0.07	14.93	(12.61–17.46)
Uterine weight, intact mice	1	40	0.14	13.73	(11.20–16.83)
	2	37	0.31	11.65	(7.28–18.52)
	3	39	0.29	11.06	(7.21–16.97)
Ventral-prostatic weight, hypophysectomized rats	1	18	0.08	11.49	(9.66–13.67)
	2	20	0.14	11.36	(7.69–17.39)
	3	20	0.15	11.71	(8.57–15.99)
Testicular weight (same animals)	1	18	0.20	12.36	(8.14–18.76)
	2	20	0.23	17.28	(8.86–33.70)
	3	20	0.66	13.64	(3.50–53.10)

* No. = assay number; N. = total number of animals; λ = index of precision; R.P. = relative potency, or units per milligram, 1 unit being the activity of 1 mg. of male HPG.

† P = 0.95.

References to statistical methods are given in a previous paper (1).

RESULTS

Table 2 shows the relative potencies of preparation B in terms of A. The individual estimations of relative potency are within or reasonably close to the confidence limits of all the assays. The unweighted mean relative potency for the 14 assays was 12.83, and the mean confidence limits were 9.12 to 20.04. The mean relative potency of 12.83 is approximately 30 times greater than its standard error ($\sqrt{\Sigma d^2/n(n-1)}$), another indication of the uniformity of the data. All but one estimation (testicular weight No. 2) fell well within two standard deviations of the mean value of 12.83. Therefore, it may be concluded that the relative potency of B in terms of A is the same for each of the four end points used.

TABLE 3. ASSAY OF POSTMENOPAUSAL HPG (AFTER DILUTION WITH 11.6 PARTS OF INERT MATERIAL) IN TERMS OF MALE HPG*

End point	No.	N.	λ	R.P.	Confidence limits †
Ovarian weight, intact rats	1	20	0.12	1.20	(0.92–1.66)
	2	20	0.09	1.27	(1.03–1.63)
	3	20	0.06	1.14	(1.00–1.31)
	4	20	0.07	1.25	(1.05–1.54)
	5	20	0.07	0.88	(0.74–1.04)
Uterine weight, intact mice	1	39	0.14	2.14	(1.75–2.61)
	2	40	0.13	0.84	(0.69–1.02)
	3	40	0.06	0.78	(0.71–0.85)
Ventral-prostatic weight, hypophysectomized rats	1	20	0.12	0.91	(0.64–1.29)
	2	19	0.13	1.09	(0.83–1.44)
	3	20	0.15	1.04	(0.76–1.42)
Testicular weight (same animals)	1	20	0.20	0.96	(0.54–1.71)
	2	19	0.16	1.47	(1.05–2.07)
	3	20	0.28	0.73	(0.41–1.30)

* Abbreviations are the same as in Table 2.
† P = 0.95.

Table 3 contains the data obtained when preparation B was diluted with inert material to make its specific activity the same as or close to that of A. If the dilution factor was accurate and if the dilutions were prepared accurately, the relative potency of B (dil.) in terms of A should be 1. The mean relative potency, namely B (dil.) ÷ A, was 1.12, and the mean confidence limits were 0.87 to 1.49. The mean relative potency is 11 times its standard error, and all but one of the individual relative potencies (uterine-weight assay No. 1) fell well within two standard deviations of the mean. Therefore, it may be concluded that the presence of inert material, at least in the proportions investigated in this study, is not a factor in the assay of HPG from various sources.

Table 4 shows the mean index of discrimination for all combinations of

TABLE 4. MEAN INDEX OF DISCRIMINATION FOR COMBINATIONS OF ASSAYS OF HPG

Method	Ovary	Uterus	Prostate	Testis
Ovary	—	1.07	1.13	0.91
Uterus	0.92	—	1.05	0.84
Prostate	0.88	0.95	—	0.80
Testis	1.10	1.19	1.25	—

the four assays. The average index of discrimination should approximate unity, provided the same biologically active material in two preparations is being measured by two different methods of assay. It may be noted that all values for the mean index of discrimination are close to unity. How much the index of discrimination must differ from the value 1 to indicate dissimilarity in the biologically active material of two preparations can be determined from the internal evidence of the assays. However, sufficient replicates were made during the course of these experiments to provide

TABLE 5. ASSAYS OF HPG VERSUS ITSELF*

End point	Preparation	Doses (mg.)	No.	N.	λ	R.P.	Confidence limits†
Ovarian weight, intact rats	A	20 –40	1	20	0.05	1.02	(0.91–1.14)
	A	20 –40	2	20	0.06	0.85	(0.73–0.97)
	A	20 –40	3	20	0.06	0.86	(0.74–0.98)
	A	15 –30	4	20	0.08	1.05	(0.87–1.30)
	B	1.5– 3.0	5	20	0.08	0.86	(0.79–0.90)
	♂III	15 –30	6	20	0.17	0.91	(0.18–1.39)
	♂III	15 –30	7	20	0.08	1.02	(0.95–1.22)
	♂III	15 –30	8	20	0.07	1.00	(0.95–1.01)
	B (dil.)	20 –40	9	20	0.08	1.11	(0.93–1.34)
	B (dil.)	20 –40	10	20	0.08	0.90	(0.75–1.07)
	B (dil.)	20 –40	11	20	0.17	0.99	(0.85–1.14)
Uterine weight, intact mice	A	1.9– 3.8	1	40	0.09	0.84	(0.74–0.96)
	B (dil.)	1.9– 3.8	2	40	0.09	0.98	(0.86–1.12)
	AMW	1 – 2	3	40	0.13	1.07	(0.88–1.29)

* Abbreviations are the same as in Table 2. Others are as follows: ♂III = purified preparation of male HPG (9); dil. = diluted with 11.6 parts of inert material; AMW = local standard of male HPG (10).

† P = 0.95.

another estimation of the allowable limits of fluctuation of the index. Several of the assays were conducted concurrently; therefore, it was possible to compare deviations from unity obtained with the same preparation. In addition, other preparations of HPG were tested against themselves. Table 5 shows 14 such assays of HPG conducted against itself. Five of the 14 assays were conducted on preparation A against itself, 4 on B (dil.) against itself, 1 on preparation B, 3 on a purified preparation of male HPG (7), and 1 on the male standard, AMW (10). The mean relative potency was 0.96, with mean confidence limits of 0.80 to 1.13. Since the actual relative potency is 1.0, the deviations experimentally obtained were calculated as deviations from the actual relative potency of 1.0. The stand-

ard error was 0.025 and the standard deviation was 0.0949. If normally distributed, two standard deviations will give limits of 1.19 in one extreme direction (1+0.19) and 0.81 (1−0.19) in the other. The interval between the two extremes thus will account for 95 per cent of all of the observations. As may be noted in Table 5, all estimations of relative potency were well within two standard deviations of the actual relative potency of 1.0. For 95 per cent of the observations obtained with the precision of the assays reported herein, the extreme limits of the index of discrimination are 0.68 and 1.47 (0.81 ÷ 1.19 and 1.19 ÷ 0.81). It can be seen from Table 4 that the values for the mean index of discrimination for all combinations of assays are well within these limits, and none differs significantly from unity.

COMMENT

Previous collaborative assays of HPG from men and postmenopausal women have provided suggestive evidence that the various methods of assay used were measuring the same gonadotropin or the same constant proportion of gonadotropins. Evidence of dissimilarity of biologic activity in two preparations can be afforded by the index of discrimination. However, the index of discrimination is subject to all the sources of error entering the assays of the two preparations. These errors can be cumulative, in which case the index can deviate from a value of 1 and yet not constitute evidence of dissimilarity. In the previous studies, interassay variation constituted a source of such error in addition to errors contributed by several other factors already pointed out. In the present study, two sources of error were minimized by appropriate experimental procedures. The limits of variation of the index of discrimination were empirically derived by assaying several HPG preparations against themselves. None of the 12 values (means of at least 3 replicates) for the index of discrimination obtained in the present study extended beyond these limits. Therefore, no evidence of dissimilarity was obtained. Consequently, the HPG of postmenopausal women and of men can be assayed in terms of each other by any of the four methods of assay used.

Considering all of the data obtained thus far by the method of quantitative assays, it appears unlikely that the question of theoretic differences in composition of HPG (namely, that urine from one source contains more or less follicle-stimulating hormone [FSH] or luteinizing hormone [LH] than does urine from another source) will be solved by further studies of this kind. If indeed the method using ventral-prostatic weight in the hypophysectomized rat measures only LH, and if the testicular-weight method in hypophysectomized rats measures FSH, it must follow that the same proportions of FSH and LH are present in preparations A and B. Thus, it is apparent that use of the ventral-prostatic weight to measure urinary LH

in normal men or postmenopausal women gives no more information than can be obtained with more convenient assays, such as the ovarian-weight method in intact rats or the uterine-weight method in intact mice, which presumably measure both FSH and LH. It remains to be shown that assays for LH by use of ventral-prostatic weight give additional information of significance in various types of endocrinopathies that is not obtained by use of technics involving the rat-ovarian and the mouse-uterine weight. Finally, with regard to the question of the unitary versus the dual nature of HPG, the present study, since it provides no evidence of dissimilarity, is interpretable in either of two ways, namely 1) that urinary HPG is one gonadotropin, or 2) that it is a mixture of two gonadotropins that exist in constant proportion (or in such unknown and variable proportions that are not discernible by conventional assays) in the urine of men and postmenopausal women. Further progress on the resolution of this question by the method of quantitative bio-assays would appear to depend on the development of specific tests of HPG that would measure only LH in the presence or absence of FSH and of tests that measure only FSH in the presence or absence of LH. Such tests with proved performance do not presently exist.

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A COMPARATIVE STUDY OF THE METABOLISM OF HYDROCORTISONE AND PREDNISOLONE*

C. A. NUGENT, M.D., KRISTEN EIK-NES, M.D. AND FRANK H. TYLER, M.D.

The Departments of Medicine and Biochemistry and the Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah College of Medicine, Salt Lake City, Utah

ABSTRACT

Intravenous infusions of the succinate esters of hydrocortisone and prednisolone were given to normal subjects and to patients with hepatic or renal disease in order to study the basis for the differing glucocorticoid effectiveness of the compounds. The plasma half-time of free 17-hydroxycorticosteroids after intravenous administration of hydrocortisone esters to normal young men was 113 minutes. The plasma half-time of free 17-hydroxycorticosteroids after intravenous administration of prednisolone esters was 204 to 241 minutes in normal subjects and in patients with renal or hepatic disease. The fact that prednisolone has a longer plasma half-time than hydrocortisone gives no clear insight into the reasons for the increased glucocorticoid effects of prednisolone compared to equivalent amounts of hydrocortisone, and their roughly similar effects on salt metabolism. The contrast in the action of the two compounds must depend on their intrinsic structures or on their different pathways of metabolism. Liver disease caused only slight delay in plasma removal of 17-hydroxycorticosteroids after prednisolone in contrast to the marked prolongation that occurs after hydrocortisone. Although patients with renal disease excreted only small amounts of free 17-hydroxycorticosteroids in their urine after intravenous prednisolone, the plasma half-time was not decreased. The rate of fall of plasma free 17-hydroxycorticosteroids after intravenous prednisolone is determined in large part by the rate of removal by organs outside the liver or kidney.

PREDNISOLONE is three to five times as potent in its glucocorticoid and anti-inflammatory actions as hydrocortisone (1). In the present investigation, an attempt was made to clarify the reason for this difference by studying plasma and urinary 17-hydroxycorticosteroid levels after the intravenous infusion of the two compounds in normal young men, in normal young women, and in patients with renal or hepatic disease. In addition, the urinary steroid excretion was compared following oral administration of hydrocortisone and prednisolone to normal subjects.

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METHODS

The hemisuccinates, or sodium succinates, of hydrocortisone and prednisolone were used. They are convenient water-soluble forms of these compounds. In every instance in this study in which weights of the steroid succinates are reported, the weight is expressed as mg. of the parent compound; *i.e.*, 100 mg. of prednisolone succinate means a weight of the hemisuccinate or sodium succinate equivalent to 100 mg. of prednisolone.

The infusions were started between 8:00 and 10:00 A.M. The subjects were either fasting or had eaten a low-fat breakfast. The steroids, dissolved in 300 ml. of 5 per cent dextrose in water, were given intravenously over a 30-minute period in a dose of 1 mg. per Kg. of body weight. Blood was drawn before the infusion, at the end of the infusion, and 60, 120, 240 and 360 minutes after the infusion was started. The volume of blood drawn for the initial specimen was 15 ml.; for the subsequent specimens it was 5 to 10 ml. Heparin was used as an anticoagulant. The blood was centrifuged within one hour after it was drawn and the plasma was separated. Plasma samples were stored in a deep-freeze at −5° F. until they were analyzed. The urines were stored in a refrigerator during the daily collections and then placed in a deep-freeze; no preservatives were used. The methods employed for the determination of plasma and urinary 17-hydroxycorticosteroids have been described in previous publications from this laboratory (2). Urinary 17-ketosteroids were determined by the method of Hamburger (3). The urinary data were corrected to a body weight of 70 Kg. The disappearance rates of free plasma 17-hydroxycorticosteroids were calculated from the concentrations of these steroids in the plasma during the last four hours of each study. During this period, the rate of removal follows first-order kinetics, *i.e.*, it is directly proportional to the plasma concentration. The time required for the concentration of plasma 17-hydroxycorticosteroids to decrease 50 per cent, termed the half-time, was calculated by the method of least squares.

RESULTS

Group I. Young men

The change in the concentration of free plasma 17-hydroxycorticosteroids following the infusion of hydrocortisone succinate into 10 normal young men is shown in Figure 1. Ages of the subjects ranged from 23 to 32, with a mean of 26 years; mean body weight was 78 Kg. The half-time of the free plasma 17-hydroxycorticosteroids was 113 minutes. The mean urinary excretion of the different fractions after steroid administration in 3 of these subjects is shown in Figure 2. The values are compared with average values for 4 normal young men whose urine was collected while they were receiving no exogenous steroids. Only a small proportion of the injected hydrocortisone was excreted in the urine in the free form, but there was a large increase in the excretion of conjugated 17-hydroxycorticosteroids.

An equivalent amount of prednisolone succinate was infused into 6 subjects from the same group of young men. A different pattern was observed: the half-time of free 17-hydroxycorticosteroids was 213 minutes (Fig. 3) and 24 per cent of the injected compound appeared in the urine as free 17-hydroxycorticosteroids (Fig. 2). The excretion of conjugated 17-hydroxycorticosteroids in the urine was only slightly increased above control levels.

Group II. Young women

Four normal young women, ranging in age from 19 to 22, with a mean age of 20 years and a mean body weight of 76 Kg., were infused on separate occasions with the succinates of hydrocortisone and of prednisolone. The plasma values are shown in Figures 4 and 5. The plasma half-time of free 17-hydroxycorticosteroids was 173 minutes after hydrocortisone succinate; this is considerably longer than the half-time in young men. The plasma half-time of free 17-hydroxycorticosteroids after prednisolone succinate was 204 minutes, a value similar to that seen in the young men following administration of this compound.

The urinary data for this group are shown in Figure 2. Compared to the young men, the young women excreted a smaller proportion of the injected steroid as 17-hydroxycorticosteroid glucuronide after either hydrocortisone or prednisolone succinate. Their excretion of free steroids was similar to that observed in the young men.

Group III. Hydrocortisone and prednisolone in liver disease

Seven patients with liver disease were studied. Four men (ages 37, 49, 51 and 71) and 3 women (ages 17, 46 and 64 years) were infused with prednisolone succinate. Three of these patients had Laennec's cirrhosis, 2 had hepatic insufficiency as a result of metastatic carcinoma, 1 had infectious

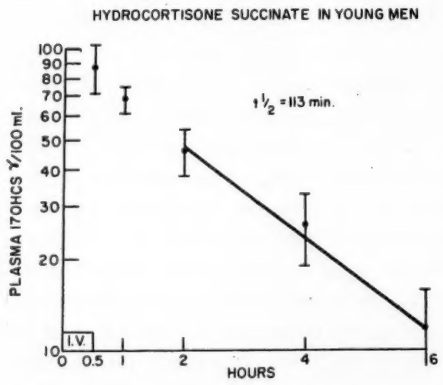

FIG. 1. Free plasma 17-hydroxycorticosteroid concentrations after infusion of 1 mg. of hydrocortisone succinate per Kg. of body weight in normal young men. The mean and standard deviations are shown. The solid line between the two-hour and six-hour values was determined by the method of least squares.

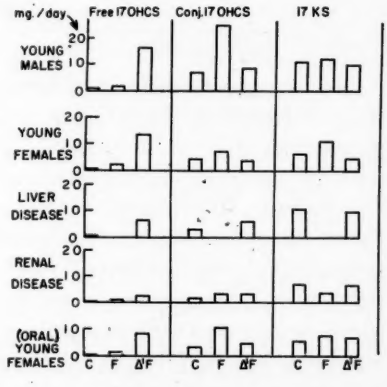

FIG. 2. Urinary 24-hour steroid excretion values (mg. per day) compared in the various groups on the control day (C), the day of hydrocortisone succinate administration (F), and the day of prednisolone administration (Δ^1-F).

hepatitis and 1 had biliary cirrhosis. Two patients were not jaundiced; their bromsulphonphthalein retentions were 25 and 31 per cent in forty-five minutes (5 mg. of dye per Kg. of body weight). The remaining patients were jaundiced, and the total serum bilirubin levels ranged from 4 mg. to 23 mg. per 100 ml. The plasma steroid values are shown in Figure 6. The half-time of the plasma free 17-hydroxycorticosteroids in these patients after prednisolone succinate was 241 minutes, a value slightly greater than in the normal subjects.

The urinary data for the patients with liver disease are shown in Figure 2. These patients excreted less free 17-hydroxycorticosteroids in their urine than did normal subjects after infusion with prednisolone, but more conjugated 17-hydroxycorticosteroids than the normal female subjects and less than the normal male subjects.

Group IV. Renal disease

Five patients with renal disease were studied. Four women (ages 42, 59, 72 and 73) and 1 man (age 55 years) were infused with the succinates of hydrocortisone and prednisolone. One of these patients had periarteritis nodosa, 1 had chronic glomerulonephritis, 1 had diabetes mellitus with Kimmelstiel-Wilson's syndrome, and in 2 cases the basis for the renal insufficiency was obscure. The blood urea nitrogen levels at the time of the study ranged from 45 to 98 mg. per 100 ml. with a mean concentration of 61 mg. Due to difficulties in the collection of the blood, inadequate numbers of plasma specimens were obtained after the hydrocortisone infusions. Consequently, the results are not presented here. The plasma steroid values after prednisolone are shown in Figure 7. The half-time of free 17-hydroxycorticosteroids was 223 minutes. This is similar to the average in the normal subjects. The urinary data for this group are shown in Figure 2. Only small amounts of conjugated or free 17-hydroxycorticosteroids appeared in the urine at any time.

Group V. Effect of oral steroids in young women

Three of the 4 young women who received steroids intravenously were also given the identical drugs by mouth. The drug (1 mg. per Kg. of body weight) was dissolved in 200 ml. of water and administered to the fasting subjects at 8:00 A.M. Plasma levels of 17-hydroxycorticosteroids were not studied. The analyses of the urinary excretions are shown in Figure 2.

Either drug by mouth produced a smaller increase in the excretion of free 17-hydroxycorticosteroids than after intravenous administration.

In general, the changes in 17-ketosteroid excretion in all the various groups were small and not consistent when data from the individual subjects were examined.

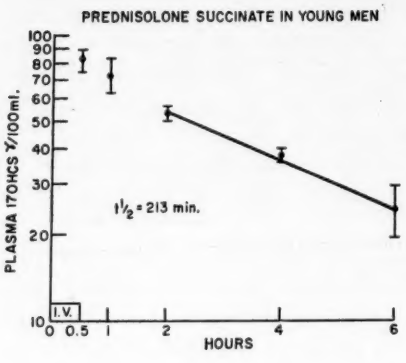

FIG. 3

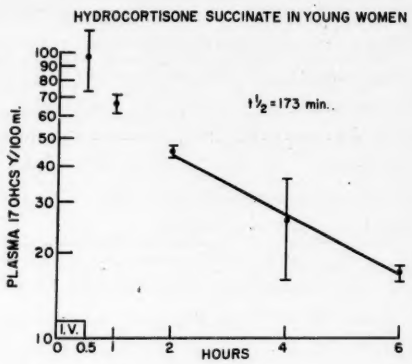

FIG. 4

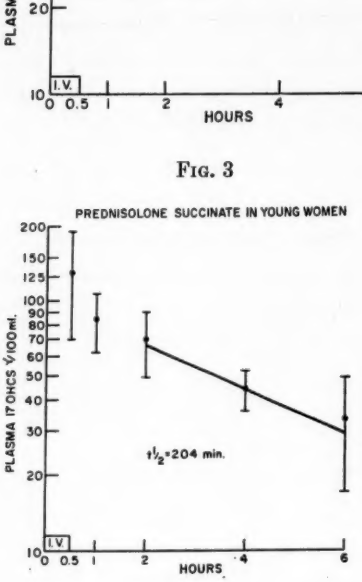

FIG. 5

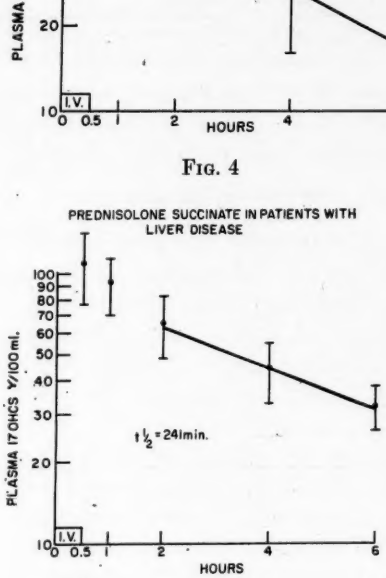

FIG. 6

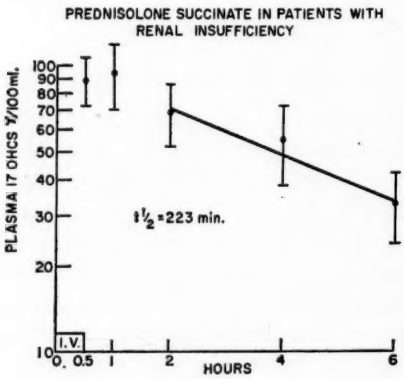

FIG. 7

DISCUSSION

The initial part of the curves of the logarithms of plasma free 17-hydroxycorticosteroid concentrations plotted against time is frequently not linear. This non-linearity is due in part to the time required for equilibration. An additional factor in the prednisolone studies is that no attempt was made to produce prior suppression of endogenous hydrocortisone production, so that the initial portion of the curve may reflect metabolism of endogenous hydrocortisone superimposed upon that of the exogenous prednisolone. Another factor which might affect the linearity of the initial part of the curve in these studies is the time required for hydrolysis of the succinate esters. In several of the studies the mean plasma concentration of 17-hydroxycorticosteroids thirty minutes after termination of the infusion was higher than the mean value for the nonconjugated steroids at the end of the infusion. However, recalculation by the method of least squares of the data given by Brown *et al.* (4) for plasma 17-hydroxycorticosteroid levels after intravenous administration of hydrocortisone to a group of normal male subjects yields a half-time of 109 minutes. This value is similar to the value of 113 minutes calculated from the data on our group of normal young men following administration of hydrocortisone succinate, and we can conclude that the time required for hydrolysis of the esters had little effect on the plasma half-time of free 17-hydroxycorticosteroids. Collings *et al.* (5) observed that in dogs the plasma half-times of free 17-hydroxycorticosteroids were slightly prolonged when the compounds were administered as esters rather than as free compounds, in contrast to our findings in human subjects.

The half-time of the plasma free 17-hydroxycorticosteroids after infusion of hydrocortisone in the 4 young women in the present study was considerably longer than the half-time in the young men. However, there was marked variation among the individual curves in the studies on the young women. Other investigators have reported that the plasma half-time of free 17-hydroxycorticosteroids after administration of hydrocortisone is prolonged in young women (6). In contrast to hydrocortisone, prednisolone succinate was removed from the plasma at a similar rate by normal young men and women.

Free plasma 17-hydroxycorticosteroid concentrations after infusion of steroid in a dosage of 1 mg. per Kg. of body weight:

FIG. 3. Prednisolone succinate in normal young men.
FIG. 4. Hydrocortisone succinate in normal young women.
FIG. 5. Prednisolone succinate in normal young women.
FIG. 6. Prednisolone succinate in patients with liver disease.
FIG. 7. Prednisolone succinate in patients with renal insufficiency.

Ely *et al.* (7) found a plasma half-time of free 17-hydroxycorticosteroids of 192 minutes after intravenous prednisolone, and Sandberg and Slaunwhite (8) obtained values of 200 to 220 minutes. The increased plasma half-time of free 17-hydroxycorticosteroids after infusion of prednisolone compared with that after hydrocortisone has been amply demonstrated in the present investigation. It has been suggested that the high glucocorticoid effects of prednisolone may be due to its slow metabolism by the body. However, the fact that prednisolone has a longer plasma half-time than hydrocortisone furnishes no clear insight into the reasons for the potent glucocorticoid effects of prednisolone. The roughly similar effects on salt metabolism of equal amounts of these steroids conclusively demonstrates that difference in rate of degradation alone cannot account for their contrasting activities.

There, are however, observations which suggest that prednisolone is metabolized by pathways different from those for hydrocortisone. Vermeulen (9) found that prednisone administered orally to human subjects was excreted in the urine as prednisone, prednisolone and Δ^{1-4}-pregnadiene, 3-11-dione-17γ-20β-21-triol. Gray *et al.* (10) examined the steroids in the urine excreted by patients given prednisone and prednisolone orally, and found prednisone, prednisolone, the corresponding 20β alcohols and a compound suggestive of pregna-1:4-dien-17α:20α:21-triol-3:11-dione. There was no evidence in the studies of Vermeulen or of Gray that there was any conversion of prednisone or prednisolone to cortisone or hydrocortisone or their derivatives. Tomkins and Isselbacher (11) observed that rat liver homogenates reduced the Δ^4-3-ketone group of hydrocortisone but not that of prednisolone.

Liver disease has been shown to result in prolongation of the plasma half-time of free 17-hydroxycorticosteroids after hydrocortisone. Brown *et al.* (4) showed that this prolongation was correlated with bromsulfonphthalein retention. The patients reported here with liver disease were on the average older than the control subjects. In a limited number of intravenous infusions of prednisolone into subjects who were 40 to 50 years old, we have found no difference in the half-time of free 17-hydroxycorticosteroids compared to that in normal young subjects. The fact that after prednisolone the half-time of free 17-hydroxycorticosteroids in patients with liver disease was only slightly greater than in the control group of normal subjects suggests either that the hepatic capacity to metabolize prednisolone is usually little affected by liver disease or, more reasonably, that the liver is not quantitatively as important a site of degradation of circulating prednisolone after its intravenous administration as in the case of hydrocortisone. Tomkin and Isselbacher's data (11) would favor the latter view.

Subjects with liver disease excreted slightly less prednisolone in a free form in their urine than did the normal subjects. Renal function studies (12) on patients with liver disease of a severity comparable to that in our group revealed only modest reductions in glomerular and tubular function and in total renal blood flow. One would not expect these slight changes in kidney function to account for the small proportion of free 17-hydroxycorticosteroids excreted by the patients with liver disease after administration of prednisolone succinate. These findings might be due to abnormal binding of plasma "free" 17-hydroxycorticosteroids, but we have no evidence to support this possibility.

The marked increase in urinary excretion of free 17-hydroxycorticosteroids after administration of prednisolone has been noted by several investigators (7, 8). The young men excreted roughly one-quarter of the infused prednisolone in a free form in the urine. One might expect, therefore, that urinary excretion would be an important determinant of the half-time of free plasma 17-hydroxycorticosteroids. However, this does not seem to be the case. The patients with renal insufficiency excreted less than 4 per cent of the infused prednisolone succinate in the free form in their urine, whereas the plasma half-time of free 17-hydroxycorticosteroids was not prolonged. This suggests that the alternative metabolic pathways of prednisolone are quantitatively of great importance. The rate of fall of plasma free 17-hydroxycorticosteroids after infusion of prednisolone probably is determined in large part by the rate of destruction by organs outside the liver or kidney.

Examination of the urinary data after oral administration of hydrocortisone and prednisolone succinates to young women shows that, although there was little change in the excretion of 17-ketosteroids or conjugated 17-hydroxycorticosteroids compared to that after intravenous administration, there was a definite decrease in the total amount excreted in the free form following oral administration. This might be due to destruction of free prednisolone by the liver. When the liver is bypassed by an Eck fistula in the dog, higher blood levels of free 17-hydroxycorticosteroids can be achieved by oral administration of hydrocortisone than in dogs without an Eck fistula (13). The results of the studies on patients with liver disease suggested that the liver was not quantitatively an important site for the degradation of intravenously administered prednisolone. Conceivably, after oral administration and absorption of prednisolone directly into the portal vein, the direct perfusion of this blood through the liver might result in greater hepatic destruction of prednisolone than when the drug is given in a systemic vein. The decrease in the amount of 17-hydroxycorticosteroids excreted in a free form after oral administration of prednis-

olone might also result from the action of bacteria or digestive enzymes on the compound, or from incomplete absorption by the gastro-intestinal tract.

Acknowledgments

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URINARY 17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS IN CASTRATED *VS.* INTACT MEN: SUPPLEMENTARY COMPARISONS IN FEEBLE-MINDED *VS.* MENTALLY NORMAL MEN*

JAMES B. HAMILTON, PH.D.†, LEITHA D. BUNCH, M.A.‡ AND GORDON E. MESTLER†

ABSTRACT

The daily urinary excretion of 17-hydroxycorticosteroids (17-OHCS) and 17-ketosteroids (17-KS) was studied in 52 castrated men and 31 intact men, 23 to 81 years of age; all were in good health and living under a similar regimen in the same institution for the feeble-minded. Mean values for 17-KS tended to be lower in castrated than in intact men, and were markedly so when calculated per Kg. of body weight. The degree of variation in 17-KS excretion from subject to subject was greater in castrated than in intact men. In a few eunuchs, the titers of 17-KS were normal or elevated. Mean titers of 17-OHCS were similar in castrated and intact men at all ages. Age at castration or duration of the castrate state did not influence values for 17-OHCS or 17-KS excretion or the relative proportions of these two types of steroid. The relationship between titers of 17-OHCS and 17-KS in the same subjects was significant, both in the eunuchs and in the intact men. Among young adult intact males, the daily values for urinary 17-KS, but not 17-OHCS, were significantly lower in the feeble-minded than in those of normal mentality. With advancing age, the values for urinary 17-KS were reduced in the eunuchs, particularly when calculated per Kg. of body weight. That aging is associated with declining titers of 17-KS in eunuchs, strongly suggests that adrenocortical secretions participate to a major extent in this phenomenon.

STUDIES of urinary steroids in men after removal of the testes are pertinent to several broad problems such as the relationship of testicular secretions to the lesser viability of males than of females and the

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† Department of Anatomy, State University of New York, College of Medicine at New York City, Brooklyn, N.Y.

‡ The H. L. Snyder Memorial Research Foundation, Winfield, Kansas.

interrelationships between gonads and adrenals, especially with regard to the question of whether or not adrenocortical functions become augmented after castration, as has been found in some rodents.

Urinary steroids of eunuchs, unlike those of intact males, are not derived from varying mixtures of testicular and adrenocortical secretions. After orchiectomy the titers of 17-hydroxycorticosteroids (17-OHCS) and 17-ketosteroids (17-KS) appear to represent metabolites that originate predominantly from adrenocortical secretions. Values for 17-OHCS and 17-KS in eunuchs are reduced almost to nil following bilateral adrenalectomy and in some cases of Addison's disease (1–3) and may be elevated in patients with hyperfunction of the adrenal cortices as a result of tumors or Cushing's disease (4). Even in intact men, measurements of 17-OHCS are accepted (3–5) as good indices of the adrenocortical secretion of corticoids.

Previous investigations of the influence of castration upon titers of urinary 17-KS have been limited to small groups of subjects (3, 6), mostly old or ill with cancer, and the excretion of 17-OHCS after orchiectomy has apparently not been studied. The present investigation involved 52 eunuchs selected to cover a range of age from 24 to 79 years. The subjects were in good health as determined by physical examination and analyses of blood and urine. Castration was carried out for legal reasons and not because of illness, a matter of importance in view of the lowering of 17-KS titers by illness (7).

The present investigation was also designed to test the effect of aging upon titers of 17-OHCS and 17-KS in eunuchs as compared with a control

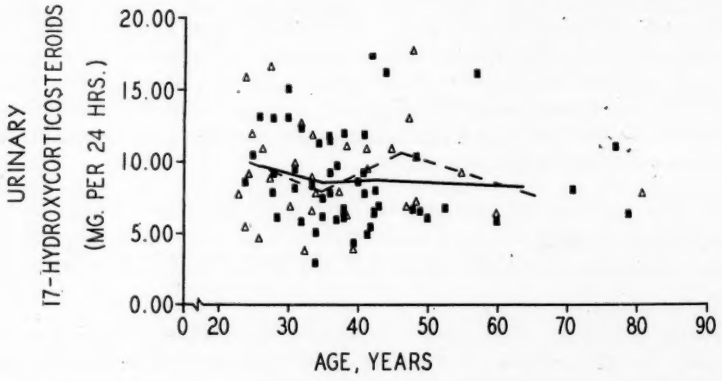

FIG. 1. Titers of urinary 17-hydroxycorticosteroids according to age. Black squares and the solid line represent eunuchs. Open triangles and the broken line represent the control series of intact men. The lines are based on the averages for ages 20–29, 30–39, 40–49, and 50 years or more.

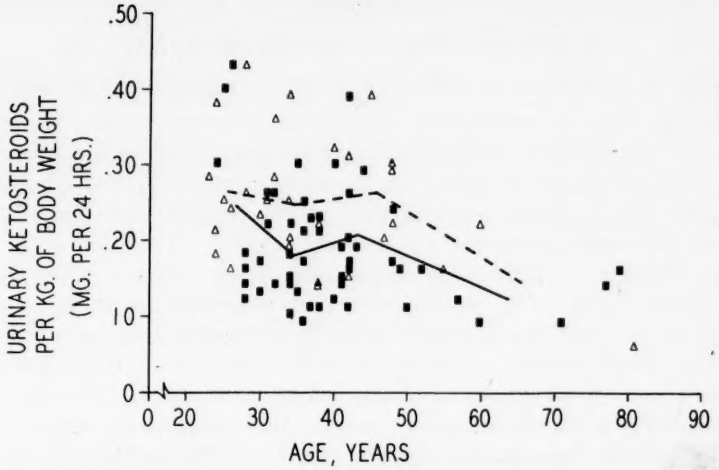

FIG. 2. Titers of urinary 17-ketosteroids according to age. Values are lower in eunuchs than in the control series of intact men and tend to decrease with age in both groups. Symbols as in Figure 1.

series of intact males. Since the eunuchs were inmates of a home for the feeble-minded, the control series was chosen from the same institution; it consisted of 31 intact males comparable in ethnic background, range of age, and routine of life, including access to the same diet.

Supplementary data for a series of 30 intact males who were not feeble-minded were obtained to ascertain if values for steroids tended to be low in the feeble-minded subjects under study. In intact males the growth of a secondary sex character, the beard, has been found (8) to be more limited in the feeble-minded than in the mentally normal. The intact men of normal mentality were not institutionalized but lived in the same community and were similar in age and ethnic status to the feeble-minded subjects.

MATERIALS AND METHODS

The titers of urinary steroids according to the age of the eunuchs and the control intact males are shown for each subject in Figures 1 and 2. Data for intact males who were not feeble-minded are presented in Table 5.

Collections of urine for twenty-four consecutive hours were obtained from each subject on 2 to 7 occasions (mean, 3.1). Separate extractions and assays of each collection were made in duplicate.

For determination of 17-OHCS, the urines were processed using the method of hydrolysis reported by Glenn and Nelson (9) and the extraction and color reaction described by Silber and Porter (10). 17-KS excretion was determined by the method of Masuda and Thuline (11).

RESULTS

Feeble-minded Males—Castrated versus Intact

Variation in urinary steroid values in the same subject, and from subject to subject

Table 1 shows the limited extent of variation in values for 3 separate collections and assays of urine from the same subjects. These data sum up variations due to technique and fluctuations from time to time in the amount of steroids excreted.

The variation in titers in the same subject, as tested in terms of the standard deviation for 10 castrated and 8 intact men (Table 1), was slightly less for 17-KS than for 17-OHCS but the difference was not significant ($P > .10$). Much greater constancy of 17-KS than of 17-OHCS excretion had been anticipated, since the daily excretion of 17-OHCS varies considerably with the degree of stress upon the subject (12); whereas the excretion of 17-KS is more stable (13). The relative uniformity of repeated determinations of 17-OHCS in the present study may have been due in part to an almost uniform pattern of life in a protected environment and to the feeble-minded status of the subjects.

The variation in titers of 17-KS from subject to subject, as determined for 19 castrated and 38 intact men 30 to 49 years old, was greater ($P = .002$) in eunuchs than in their intact controls. The variation in titers of 17-OHCS was slightly but not significantly ($P = .20$) less in castrated than in intact men. Calculated as a percentage of the amount excreted per day, the degree of variation was also significantly greater in the eunuchs than in the intact controls as regards 17-KS (diff. $= 20.3 \pm 5.04$) but not 17-OHCS (diff. $= 2.8 \pm 5.62$).

17-OHCS excretion

Absolute values (mg. per twenty-four hours). The eunuchs and the control group of intact males had similar titers when the series was considered as a whole (Fig. 1) and in comparisons of age groups (Table 2).

Aging was not accompanied by a significant decline in excretion of 17-OHCS in either the castrate ($P = .52$) or the intact ($P = .75$) group. In comparisons of groups 23 to 39 years old with those 40 to 81 years old, the mean values also were not significantly different, being 8.70 mg. and 8.55 mg. respectively in eunuchs, and 8.86 mg. and 9.76 mg. respectively in the intact controls.

Relationship to body weight. In eunuchs this relationship was significant, largely because of the high correlation for men less than 40 years old (Table 3). In the intact controls (Table 3) there was a suggestion ($P = .05$) of significance for the series as a whole, again with a higher correlation in the

TABLE 1. VARIATION IN VALUES FOR 17-HYDROXYCORTICOSTEROIDS (17-OHCS) AND 17-KETOSTEROIDS (17-KS) IN 3 SEPARATE COLLECTIONS AND ASSAYS OF URINE FROM THE SAME SUBJECTS

Steroids	Group	Age (yrs.)	$\overline{X}$*	S.D.†	C.V.‡
17-OHCS	Eunuchs	26	12.990	3.0432	23.4
		30	14.787	1.8380	12.4
		32	5.723	2.114	36.9
		34	4.867	0.0424	8.7
		35	7.307	3.8703	53.0
		36	11.567	4.0540	35.0
		40	8.517	0.5941	7.0
		42	17.186	4.1746	24.3
		42	6.430	3.3588	52.2
		71	7.807	0.3475	4.5
					Av. *25.7*
17-OHCS	Intact controls	27.5	8.637	1.4802	17.1
		32.5	2.993	0.5702	19.1
		33.5	6.440	1.0722	16.6
		33.5	11.650	6.0475	51.9
		37.5	7.673	2.5314	33.0
		39.5	3.723	0.5032	13.5
		45	10.593	1.8793	17.7
		60	6.240	1.6889	27.1
					Av. *24.5*
17-KS	Eunuchs	26	27.156	7.4908	27.6
		36	20.350	6.3594	31.2
		32	8.577	1.8910	22.0
		34	7.483	0.3310	4.4
		35	15.640	4.2650	27.3
		36	20.350	6.3594	31.2
		40	21.156	0.9984	4.7
		42	22.120	7.5190	34.0
		42	10.383	4.8731	46.9
		71	8.493	0.8157	9.6
					Av. *22.5*
17-KS	Intact controls	27.5	15.957	3.0284	19.0
		32.5	17.70	5.551	31.4
		33.5	16.003	0.4215	2.6
		33.5	24.780	8.8420	35.7
		37.5	10.250	2.1035	20.5
		39.5	18.33	3.630	19.8
		41.5	19.43	2.40	12.4
		45	19.840	3.9661	20.0
		47	13.50	1.054	7.8
		60	11.763	1.2680	10.8
					Av. *18.0*

* Mean value. † Standard deviation. ‡ Coefficient of variation.

TABLE 2. URINARY STEROID TITERS ACCORDING TO AGE GROUPS

Age group (yrs.)	Status	No. of subjects	17-hydroxycorticosteroids (mg.)								17-ketosteroids (mg.)							
			Per 24 hours				Per Kg. body weight				Per 24 hours				Per Kg. body weight			
			Mean	S.D.	C.V.	P	Mean	S.D.	C.V.	P	Mean	S.D.	C.V.	P	Mean	S.D.	C.V.	P
24 –28.5	Castrated	7	9.61	2.603	27.1	.88	0.15	0.033	22.3	.90	16.11	9.172	56.9	.88	0.25	0.129	52.1	.71
23 –27.5	Intact	9	9.89	4.108	41.5		0.15	0.048	32.1		16.74	6.316	37.7		0.27	0.088	33.0	
30 –39.5	Castrated	23	8.43	3.106	36.8	.64	0.12	0.032	25.2	.80	12.40	6.226	50.2	.20	0.18	0.059	33.0	.01
30.5–39.5	Intact	12	7.92	2.8453	35.9		0.13	0.058	45.1		15.02	4.155	27.7		0.25	0.078	31.6	
40 –49	Castrated	15	8.68	3.688	42.5	.25	0.14	0.067	47.4	.25	13.00	4.725	36.3	.16	0.21	0.074	35.8	.12
41.5–48.5	Intact	7	10.62	3.667	34.5		0.17	0.052	30.1		15.88	3.440	21.7		0.27	0.080	30.1	
50 –79	Castrated	7	8.42	3.744	44.5	.72	0.12	0.034	28.9	.86	8.49	1.868	22.0	.90	0.12	0.031	24.7	.50
55 –81	Intact	3	7.62	1.356	17.8		0.12	0.153	124.5		8.66	3.471	40.1		0.15	0.080	54.6	
All ages	Castrated	52	8.66	3.239	37.4	.60	0.13	0.045	34.2	.25	12.55	6.101	48.6	.05	0.19	0.080	42.3	.005
	Intact	31	9.07	3.434	37.9		0.14	0.053	36.3		15.10	5.025	33.3		0.25	0.086	34.7	

TABLE 3. ANALYSIS BY AGE GROUPS OF THE RELATIONSHIP OF 17-HYDROXYCORTICOSTEROIDS (17-OHCS) AND 17-KETOSTEROIDS (17-KS) TO ONE ANOTHER AND TO BODY WEIGHT

Age group (yrs.)	Status	No. of subjects	Correlation of body weight (Kg.) with				Correlation of 17-OHCS with 17-KS	
			17-OHCS (mg./24 hrs.)		17-KS (mg./24 hrs.)			
			r	P	r	P	r	P
24–39.5	Castrated	30	+.730	<.001	+.586	<.001	+.700	<.001
23–39.5	Intact	21	+.321	.17	0	—	+.450	.04
40 –79	Castrated	22	0	—	+.468	.03	+.593	.006
41.5–81	Intact	10	0	—	0	—	+.440	.20
All ages	Castrated	52	+.507	<.001	+.568	<.001	+.637	<.001
	Intact	31	+.369	.05	+.011	>.90	+.464	.009

younger (23–39 years) than in the older (40–81 years) group. A relationship has been described previously for intact subjects (14) but no data have been reported for eunuchs.

In individual subjects, titers of 17-OHCS bore no relationship to body fat assessed in terms of the thickness of several skinfolds, including one at the midpoint of the posterior surface of the upper arm.

Relative values (*mg. of 17-OHCS per Kg. of body weight*). There were no significant differences between the eunuchs and the intact controls (Table 2) and no change with aging ($P > .41$) in either group.

17-KS excretion

Absolute values (*mg. per twenty-four hours*). Mean (Table 2) and median values tended to be lower at all ages in the castrated men than in the intact controls, but the difference was of questionable significance ($P = .05$) in the series as a whole.

In old subjects the mean values for castrated and for intact men were nearly alike (Table 2). Testicular function is markedly reduced in old men (3, 15–17) and titers of 17-KS are as low in them as in young eunuchs (16). Orchiectomy does not materially reduce the already low values for 17-KS in old men (3, 18).

In the 20–29 year age group the mean titers were also similar in castrated and in intact men, partly as a result of the exceptionally high values for certain young eunuchs. In this age group, 6 of the 9 subjects who excreted more than 20 mg. per day were eunuchs.

With advancing age, the titers of 17-KS underwent a reduction that approached significance in both castrate ($P = .03$) and intact ($P = .04$) control groups. In intact men a decrease with aging has been reported previously for total urinary 17-KS (3, 17, 19), for both alpha-hydroxy and beta-hydroxy fractions of 17-KS (19), for the androgenic activity of the alpha-hydroxy fraction of 17-KS (15), and for chromatographically-separated steroids derived chiefly from testicular secretions (20, 21). With aging, a more drastic reduction in 17-KS than in 17-OHCS has been described (14) in intact subjects. The new finding, that a reduction in 17-KS with aging occurs in eunuchs, is proof that extratesticular tissues are involved in this phenomenon.

Relationship to body weight. This was significant ($P < .001$) in eunuchs (Table 3) but not in the small group of intact controls, although a positive correlation has been observed in other studies of intact subjects (14, 19). That the correlation between 17-KS excretion and body weight exists in eunuchs is proof that extragonadal tissues participate in this phenomenon; the concept of a relationship of the adrenal cortices to body weight is further supported by the fact that the urinary titers of 17-OHCS in eunuchs are related to body weight. In a previous study of intact males (15), the correlation of body weight with titers of 17-KS disappeared in old age and the relationship of body weight to titers of urinary androgens became insignificant still earlier, that is, by middle age. In the present study of eunuchs, the correlation of body weight with values for 17-KS or 17-

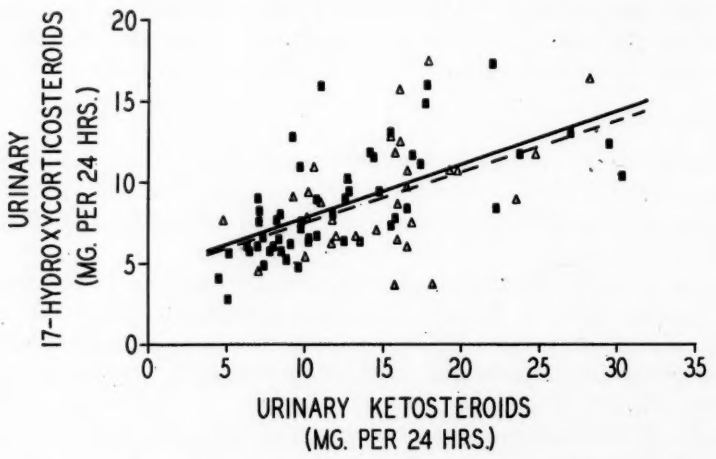

FIG. 3. The relationship between urinary 17-OHCS and 17-KS is shown separately for eunuchs and the control series of intact males. Symbols as in Figure 1. The line for each series is based on all subjects regardless of age.

TABLE 4. THE AGE OF THE SUBJECTS AT TIME OF CASTRATION AND THE DURATION OF THE CASTRATE STATE AS RELATED TO TITERS OF URINARY STEROIDS, IN EUNUCHS 20 TO 49 YEARS OLD AT THE TIME OF STUDY

	Mean age at time of study (yrs.)	No. of subjects	17-Hydroxycorticosteroids (mg./24 hrs.)			17-Ketosteroids (mg./24 hrs.)		
			Mean	Range	Median	Mean	Range	Median
Age at castration								
12–18	35.4	22	8.9	4.1–14.8	8.7	13.7	4.6–29.6	11.8
19–33	36.3	22	8.4	2.8–17.2	7.2	12.6	5.2–30.4	11.2
Years since castration								
5–10	30.0	9	8.9	6.0–13.0	8.4	15.2	8.1–30.4	13.0
11–15	33.0	10	7.8	2.8–14.8	7.2	11.3	5.2–17.8	10.9
16–20	38.5	11	9.0	4.8–12.9	7.6	13.2	6.5–17.0	8.9
21–33	40.0	14	9.4	4.1–17.2	8.6	15.4	4.6–12.3	14.6

OHCS excretion also tended to be less in the old men than in young adults (Table 3).

Values for 17-KS per Kg. of body weight were not significantly correlated with body fat assessed in terms of thicknesses of skinfolds.

Relationship between 17-OHCS and 17-KS in the same subject

Absolute values (mg. per twenty-four hours). This relationship (Fig. 3) was significant both in the eunuchs and the intact controls (Table 3). It remained significant when the factor of age was excluded by partial correlations.

The relationship between 17-KS and 17-OHCS would be expected to be closer in castrated than in intact males, since in the former the urinary 17-KS do not include metabolites derived from testicular secretions and therefore, like 17-OHCS, are derived more exclusively from adrenocortical secretions. In keeping with this expectation, the coefficients of correlation were higher in the castrated than in the intact males in both the younger and the older groups (Table 3). The difference between coefficients of correlation in the series of castrated and intact males was not statistically significant, however, for any age group or for all ages.

The ratio of 17-KS to 17-OHCS decreased with age, since titers of 17-KS underwent a greater decline than those of 17-OHCS. The reduction in this

TABLE 5. URINARY STEROID VALUES IN INTACT MEN 22 TO 42 YEARS OF AGE. COMPARISON OF THE MENTALLY NORMAL AND THE FEEBLE-MINDED

Mental status	No. of subjects	Mean age (yrs.)	17-Hydroxycorticosteroids (mg.)								17-Ketosteroids (mg.)							
			Per 24 hrs.				Per Kg. of body wt.				Per 24 hrs.				Per Kg. of body wt.			
			Mean	S.D.	C.V.	P.	Mean	S.D.	C.V.	P.	Mean	S.D.	C.V.	P.	Mean	S.D.	C.V.	P.
Feeble-minded	23	32	8.9	3.4	38	.20	0.14	0.05	37	.40	15.7	5.1	32	.01	0.25	0.08	32	.90
Normal	30	30	9.9	2.2	22		0.13	0.03	23		19.1	4.4	23		0.25	0.07	27	

ratio with aging approached significance in eunuchs (P = .04) but not in the intact controls (P = .70).

Relative values (mg. per Kg. of body weight). When corrected for body weight, the relationship between 17-KS and 17-OHCS was significant in both the castrated (r = +.610; P < .001) and the intact (r = +.473; P = .009) groups.

Lack of relationship of titers of urinary steroids to age at time of castration or to duration of the castrate state

Analysis was restricted to the 44 eunuchs who were 20 to 49 years of age, since the excretion of 17-KS was low in the more aged subjects. Titers of 17-KS and of 17-OHCS, either in absolute values or relative to body weight, were not related significantly to the subject's age at the time of castration or to the duration of the castrate state (Table 4). The comparative amounts of 17-OHCS and 17-KS were also uninfluenced.

Feeble-minded versus Mentally Normal Intact Males

Among intact feeble-minded men as compared with intact men of normal mentality (Table 5), the titers of urinary 17-OHCS were slightly although not significantly lower, but the titers of 17-KS were definitely lower (P = .01). Calculated on the basis of body weight, the difference between the two groups tended to disappear.

DISCUSSION

Mean titers of 17-KS in the eunuchs of this series were higher than those reported for the few noncancerous castrated males previously investigated (3, 6, 22, 23). This might be due in part to differences in techniques and to the small number and poor state of health of many of the subjects in previous studies. Another, and a major, reason for the high mean values in the present series was the presence of several eunuchs with a high output of 17-KS, as verified in repeated collections of urine. The excretion of 17-KS by these and other eunuchs (21 in all), and by 8 intact men in the control series, was then explored with a different technique (6); the values for the eunuchs (but not for the intact males) were again found to be higher than reported in the literature and to be especially elevated in the same eunuchs who had high titers when studied by the routine technique.

Because of elevated titers of 17-KS in some eunuchs and low titers in the control series of feeble-minded males, the difference in steroid excretion between these castrated and intact males is less than in previous reports. The average amount of 17-KS excreted by our 38 eunuchs who were 30–40 years of age was 82 per cent of that excreted by the 19 men of the same

age in the intact control series, whereas in the data of Callow *et al.* (23), Johnsen (6), and others (3), the mean value for eunuchs tended not to exceed 55 per cent of that for intact men of somewhat comparable age.

Chromatographic studies (24), carried out by Johnsen's method (6) but with findings for eunuchs that differ in some regards from those of Johnsen, indicate that most eunuchs excrete subnormal amounts of fractions which include androsterone and etiocholanolone, and that a small percentage of eunuchs excrete abnormally large amounts of fractions containing dehydroisoandrosterone. Thus the decrease in some fractions of 17-KS in young adult eunuchs may be obscured by increments in other fractions. Obviously, estimates (22) of testicular secretions on the basis of differences in total 17-KS excretion between castrated and intact men are unreliable.

The rate of decline in 17-KS excretion with aging was as drastic in eunuchs as in the control group of intact men. Therefore, the reduction in 17-KS excretion with aging in intact men cannot be ascribed solely to waning of testicular secretions. Since the adrenal cortices are presumed to be the predominant source of 17-KS in the absence of the testes (1, 2), the reduction in urinary titers of 17-KS upon aging of eunuchs strongly suggests a marked decrease in this function of the adrenal cortex with advancing age. The decline with age is less for 17-OHCS than for 17-KS, which suggests that the facet of adrenocortical function pertaining to 17-OHCS total or intermediary metabolism, is better preserved. A lesser reduction with aging for C_{19} 11-oxygenated steroids as compared to 17-KS has been described in intact subjects (17, 20); the present data show that this phenomenon is maintained in the absence of the testes and despite alterations in the proportion of total 17-KS contributed by certain chromatographically separated fractions (6, 24).

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THE SERUM PATTERN OF THYROID HORMONES IN EUTHYROIDISM AND HYPERTHYROIDISM

WALTER L. ARONS, M.D.* AND JERROLD D. HYDOVITZ, M.D.†

The Endocrine Section of the Medical Clinic, William Pepper Clinical Laboratory and Department of Radiology of the Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

ABSTRACT

The serum pattern of thyroid hormones in 26 euthyroid and 28 hyperthyroid subjects was studied by paper chromatographic techniques after the oral administration of radioactive iodine. Serum samples were drawn at intervals varying from one to 144 hours after the administration of radioiodine. Thyroxine was the major hormone demonstrated in every case. Triiodothyronine was detected in 3 of the 26 euthyroid subjects and in 10 of the 28 hyperthyroid patients. The presence of triiodothyronine in the serum did not seem to be associated with radiation effect on the thyroid gland nor could the existence of this hormone in the serum of the hyperthyroid subjects be correlated with any of the various parameters of thyroid function or the clinical severity of the thyrotoxicosis. No definite evidence of circulating monoiodotyrosine or diiodotyrosine was found, although in several cases an unidentified zone of labeled iodine was noted in the chromatograms at the DIT-MIT junction area. The acetic analogues of thyroxine and triiodothyronine and the compounds 3:3′-dliodothyronine and 3:3′:5′-triiodothyronine could not be detected in the serum of several euthyroid and hyperthyroid patients.

UNTIL six years ago thyroxine (3:5:3′:5′-tetraiodothyronine) was the only identified circulating thyroid hormone in man (1). In 1952 Gross and Pitt-Rivers (2) demonstrated the presence of 3:5:3′-triiodothyronine in the serum of man. Benua and Dobyns (3, 4) have reported on the serum pattern of thyroxine, triiodothyronine and other iodine-containing substances in a series of euthyroid and thyrotoxic subjects. Within the last three years the metabolic activity of the acetic-acid analogues of thyroxine and triiodothyronine has been demonstrated both *in vitro* (5) and in man (6, 7). Certain tissues such as kidney (8) and brain (9) have been shown to be capable of converting thyroxine and triiodothyronine to their acetic-acid analogues. Also, Roche and his colleagues (10) have recently described 2 other iodothyronines (3:3′-diiodothyronine and 3:3′:5′-triiodothyronine)

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* Present address: The Medical Plaza-Stanford, Palo Alto, California.

† Present address: Montefiore Hospital, Pittsburgh, Pennsylvania.

in the serum of rats. The possible existence of the acetic-acid analogues of thyroxine and triiodothyronine and of 3:3′-diiodothyronine and 3:3′5′-triiodothyronine in the serum of man is as yet unsettled. The present study was undertaken to determine the serum pattern of thyroid hormones in euthyroidism and hyperthyroidism in man, with particular reference to the presence of 3:5:3′-triiodothyronine and of the acetic-acid analogues of thyroxine and triiodothyronine. An attempt was also made to identify 3:3′-diiodothyronine and 3:3′:5′-triiodothyronine in the serum of man.

METHODS

Twenty-six euthyroid and 28 hyperthyroid patients were studied. The dose of I^{131} administered to the euthyroid patients varied from 1.5 to 20 millicuries (mc.), whereas the range was from 1 to 10 mc. for the hyperthyroid patients. The euthyroid subjects, ranging in age from 29 to 76 years, were given radioiodide for the relief of symptoms of angina pectoris, intractable heart failure or pulmonary emphysema. The hyperthyroid patients ranged in age from 32 to 73 years and each had a diffuse toxic goiter. None of these patients had severe malignant ophthalmopathy. Diagnoses of thyrotoxicosis were made on the basis of the clinical picture in combination with results of the usual thyroid function tests (thyroidal I^{131} uptake, BMR, and serum protein-bound iodine and cholesterol levels). The radioiodide used in these studies (carrier-free NaI) was obtained from the Oak Ridge National Laboratories.

Serum samples were drawn serially at 1, 3, 6, 24, 48, 72, 96 and up to 144 hours after the oral administration of the radioiodide. In the individual case, however, usually only three or four of these serial samples were taken. Each serum sample of approximately 6 ml. was first slurried with approximately 0.5 Gm. (dry weight) of an anion exchange resin[1] in order to remove excessive amounts of inorganic radioactive iodide which would tend to obscure more important adjacent peaks of radioactivity. This anion exchanger removed little or no thyroxine or triiodothyronine from the serum, as determined by I^{131}-labeled radiothyroxine and radiotriiodothyronine studies, but its use was discontinued during the latter half of the study for fear that it might remove other unknown circulating iodinated materials that were of interest. A 4-ml. aliquot of serum was extracted at pH 2, once with a double volume of n-butanol, and twice with equal volumes of n-butanol. The butanol extracts were then combined and evaporated to dryness under an air stream at 50° C. The residue was taken up in 6 to 10 drops of 90 per cent ethanol and 27 per cent ammonium hydroxide (9:1), and approximately 10 μg. each of carrier thyroxine, 3:5:3′-triiodothyronine,[2] monoiodotyrosine,[3] diiodotyrosine and potassium iodide added. In some experiments carrier amounts of the acetic-acid analogues of thyroxine[2] and triiodothyronine[2] were added also. This solution was then applied to a 3- or 4-cm. line on Whatman #1 filter paper for separation by paper chromatography. Serum extracts were run in duplicate in ascending n-butanol:2*N* NH_4OH [1:1] or n-butanol:dioxane:2*N* NH_4OH [4:1:5], and descending tertiary amyl-alcohol: 2*N* NH_4OH [1:0] systems. Chromatograms were allowed to develop over a 24-hour

[1] Amberlit IRA 400 in the chloride cycle (Rohm and Haas Co., Philadelphia, Pa.).

[2] Generously supplied by Smith Kline & French Laboratories of Philadelphia, Pa. for these studies.

[3] Furnished by Dr. Rosalind Pitt-Rivers, National Institute for Medical Research, Mill Hill, London, England.

period to a length of approximately 30 cm. and were then dried and sprayed with diazotized sulfanilic acid and 1 per cent palladium chloride (iodide localization), to identify the carrier sites. The chromatograms were divided into 0.5-cm. horizontal strips for radioactivity measurement in a scintillation well counter. Each strip was counted for one minute and the radioactivity of the known carrier sites was determined. Activity in two or more adjacent strips of over 50 counts per minute above the baseline value (which was approximately 250–300 counts per minute) was considered to constitute a significant peak.

Two-dimensional chromatography as recommended by Roche *et al.* (10) was utilized in some subjects in an attempt to identify 3:3′-diiodothyronine and 3:3′:5′-triiodothyronine. In these instances ascending butanol:2*N* NH_4OH constituted the first solvent system, and ascending methanol:0.2*M* ammonium acetate [2.5:1] at 4° C. the second system.

RESULTS

Euthyroidism

The typical radiopaperchromatographic findings in the 26 euthyroid subjects are shown in Figure 1. In every instance, no matter what the time interval after the administration of I^{131}, the main concentration of radioactivity was in the thyroxine (T_4) area. This peak appeared in significant amounts as early as one hour after the administration of I^{131}. Definite radioactivity in the 3:5:3′-triiodothyronine (T_3) area was found in only 3 of the 26 euthyroid subjects. One of these was a former hyperthyroid patient who had undergone subtotal thyroidectomy. In all 3 instances, the triiodothyronine radioactivity in serum appeared no earlier than twenty-four hours. In our experience it has been difficult to demonstrate triiodo-

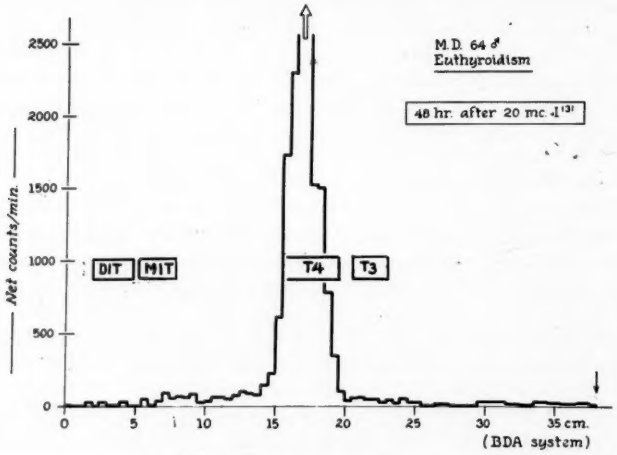

FIG. 1. Chromatogram of a sample of serum from a euthyroid subject. Radioactivity is confined to the thyroxine (T_4) zone.

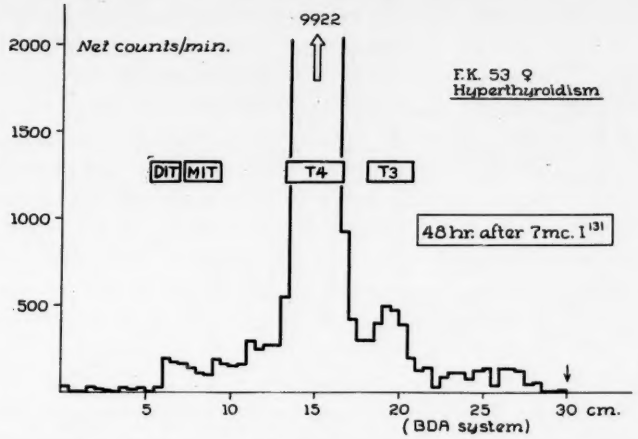

FIG. 2. Chromatogram of a sample of serum from a hyperthyroid patient. In addition to a large thyroxine (T_4) peak, triiodothyronine (T_3) radioactivity is also evident.

thyronine in the sera of euthyroid persons, in contrast to our findings in hyperthyroidism. Because of the infrequency and the small area of the triiodothyronine peaks superimposed on the usual baseline radioactivity, the calculation of the ratio of I^{131}-labeled thyroxine to I^{131}-labeled triiodothyronine could not be determined with accuracy in this group of patients.

In no euthyroid patient was there evidence of circulating monoiodotyrosine (MIT) or diiodotyrosine (DIT), but in 2 cases there was a small amount of labeled iodine at the DIT-MIT junction.

Hyperthyroidism

The chromatographic findings in the 28 thyrotoxic patients demonstrated that the major concentration of radioactivity was in the thyroxine area in every instance, just as in the euthyroid subjects. The labeled thyroxine appeared in definitely measurable amounts as early as one hour after the administration of the I^{131}. In contrast, however, to the findings in euthyroidism, significant triiodothyronine radioactivity was demonstrable in the sera of 10 of the 28 hyperthyroid patients. A chromatogram demonstrating such a triiodothyronine peak is shown in Figure 2. The triiodothyronine peaks could be detected as early as six hours after the administration of the radioactive iodide. In no instance did we find more triiodothyronine radioactivity than thyroxine activity in these thyrotoxic subjects, in contrast to the experience of other investigators (4). In the hyperthyroid patients no definite radioactivity could be ascribed to the DIT or MIT areas, but in 8 instances there was significant radioactivity close to the junction of the DIT-MIT carrier sites (Fig. 3). This radioactivity, which also was seen in 2

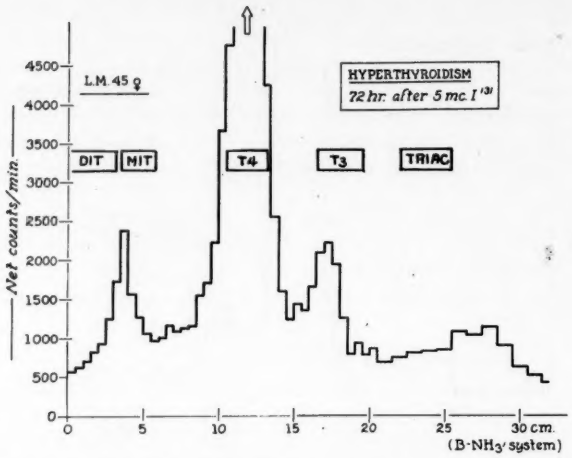

FIG. 3. In this hyperthyroid patient, in addition to thyroxine (T_4) and triiodothyronine (T_3) peaks, a peak is noted at the DIT-MIT junction. A small amount of nonspecific radioactivity is seen at the solvent front.

euthyroid subjects, did not conform exactly with either the monoiodotyrosine or diiodothyrosine areas in the single-dimension chromatographic systems employed, but tended to have an intermediate Rf. It did not appear consistently in the serum samples from the patients in whom it was present, nor was there a pattern to its fluctuations. When two-dimensional chromatography was utilized (n-butanol:2*N* NH_4OH followed by methanol:0.2*M* ammonium acetate at 4° C.) followed by radioautography, this small amount of radioactivity could not be definitely detected in the MIT or DIT areas or elsewhere, probably as a result of dispersion during the second dimension phase. The evidence would thus indicate that this radioactivity peak does not represent either circulating mono- or diiodotyrosine, but its true identity (whether a circulating thyroid secretion product or an artifact) remains unknown to us.

An attempt was made to determine the ratio of thyroxine radioactivity to triiodothyronine radioactivity in the sera of the thyrotoxic patients in whom the latter substance was present. Triiodothyronine/thyroxine ratios (of areas under peaks) varied from .05 to .12, with a mean of .08. Because of the inaccuracies previously discussed of determining the exact area of the triiodothyronine peak, these triiodothyronine/thyroxine ratios are correspondingly gross approximations.

Acetic-acid analogues

The acetic-acid analogues of thyroxine (tetrac) and of triiodothyronine (triac) were added as carriers in the chromatography of serum from 5

euthyroid and 4 hyperthyroid patients. In the solvent systems utilized (butanol:2*N* NH_4OH and tertiary amyl-alcohol:2*N* NH_4OH), tetrac runs just proximal but so closely to 3:5:3′-triiodothyronine that these systems are relatively unsatisfactory for separating these two compounds (there is less than a centimeter difference between them). On the other hand, triac has a definitely higher Rf than triiodothyronine and is well separated from it by several centimeters in both of these systems. In these 9 patients no definite radioactivity could be observed in the triac zones (Fig. 3) or the tetrac area. Similarly, upon review of all the data on our euthyroid and hyperthyroid subjects pertaining to chromatograms obtained without the use of carrier triac and tetrac, there was no evidence of radioactivity peaks at the triac and tetrac Rf's.

Attempts to identify 3:3′-diiodothyronine[4] and 3:3′:5′-triiodothyronine[4] in the serum of man were repeatedly frustrated by our inability to reproduce the chromatographic separation of these substances from thyroxine and 3:5:3′-triiodothyronine by the two-dimensional system recommended by Roche *et al.* (10). In our experiments, neither of these substances as well as thyroxine and 3:5:3′-triiodothyronine migrated in the methanol:ammonium acetate system. The reason for this discrepancy is not clear. Possibly faulty synthesis of the 3:3′- and 3:3′:5′-iodothyronines in one of the laboratories may explain these differing results. In radioautographs carried out on serum samples taken twenty-four to forty-eight hours after administration of I^{132} and subjected to two-dimensional chromatography, no radioactivity was seen at positions that would correspond to 3:3′-diiodothyronine and 3:3′:5′-triiodothyronine, as reported by Roche *et al.* (10).

DISCUSSION

Of a total of 101 chromatographic studies carried out on serum samples from 54 euthyroid and hyperthyroid subjects at intervals of one to 144 hours after administration of I^{131}, the major concentration of radioactivity was always in the thyroxine area. In no instance were we able to demonstrate more radiotriiodothyronine than radiothyroxine in serum, as has been previously reported in thyrotoxic patients (4). In the hyperthyroid patients in whom circulating radiotriiodothyronine could be demonstrated, the level of this hormone varied from one twentieth to one eighth of the level of circulating radiothyroxine. It should be pointed out that the ratio of radiotriiodothyronine to radiothyroxine in serum does not necessarily correspond to the chemical levels of these hormones, since the specific activities of these substances in serum may be different.

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[4] Synthesized and provided by Warner-Chilcott Laboratories, Morris Plains, N.J.

The infrequent occurrence of demonstrable triiodothyronine in the sera of euthyroid patients (3 out of 26) in our series is in contrast with the more frequent demonstration of triiodothyronine in the patients with thyrotoxicosis (10 of 28 cases). Although these data agree in general with those of a previous report (4), they differ with regard to the percentage of cases in which triiodothyronine could be detected. The physiologic implications of the more common incidence of triiodothyronine in the serum of hyperthyroid than euthyroid subjects are not clear. These chromatographic studies did not demonstrate the presence of any abnormal thyroid hormone in hyperthyroidism, as Plummer (11) postulated many years ago. The increased levels of circulating radiotriiodothyronine noted in some of the thyrotoxic patients would seem to indicate an increase in the true chemical level of circulating triiodothyronine in hyperthyroidism. Even in thyrotoxicosis, however, these quantities are so small as to be demonstrable by the techniques utilized in only a minority of the cases. The higher serum levels of radiotriiodothyronine demonstrated in thyrotoxicosis conceivably might be the result of more complete tagging of the iodine sites of this compound in the thyroid gland of the hyperthyroid as compared to the euthyroid patient. This is unlikely, in view of the fact that triiodothyronine pools have been found to be approximately the same size in both euthyroid and hyperthyroid glands (12). Since the serum thyroxine level, constituting approximately 85 per cent of the protein-bound iodine, is also definitely and consistently elevated in thyrotoxicosis, there is no clear-cut basis at present for implicating triiodothyronine alone as the major etiologic factor in hyperthyroidism. There is, however, evidence (some of it conflicting) to indicate that triiodothyronine may be formed in the periphery by deiodination of thyroxine (9, 13, 14) in addition to being secreted directly by the thyroid gland (15, 16, 18). The relative metabolic roles played by thyroxine and triiodothyronine in the genesis of thyrotoxicosis are, at present, in need of further elucidation.

The question arises as to whether the demonstrability of triiodothyronine in the serum was merely a function of the amount of I^{131} administered and the amount of radioactivity associated with the PBI^{131} in the chromatographed serum samples. Such a correlation would be true if the demonstration of triiodothyronine were dependent entirely upon the amount of I^{131} available to tag the triiodothyronine present in the thyroid. There was, however, no correlation between the size of the I^{131} dose or the PBI^{131} radioactivity of the chromatographed serum sample and the presence of triiodothyronine in the chromatograms. Triiodothyronine was demonstrated after as small a dose as 4 mc. given to a hyperthyroid patient, and the administration of 20 mc. to several euthyroid subjects was not followed by

demonstrable triiodothyronine radioactivity. Similarly PBI^{131} levels of 230,000 counts per minute in 4 ml. of euthyroid serum included no triiodothyronine, whereas 100,000 counts per minute in hyperthyroid serum included a definite triiodothyronine peak.

The presence of circulating triiodothyronine radioactivity in the hyperthyroid patients did not seem to be the result of radiation damage to the thyroid, since triiodothyronine radioactivity appeared as early as six hours after the administration of the radioiodine. Also, the triiodothyronine radioactivity did not increase after twenty-four hours, even when the serum was chromatographed up to 144 hours after the dose of I^{131}. One would have expected triiodothyronine radioactivity in serum to increase with elapsed time if the presence of this hormone represented a radiation effect.

An attempt was made to correlate various indices of thyroid function with the presence or absence of circulating triiodothyronine in both the euthyroid and hyperthyroid groups. No correlation was apparent between the presence or absence of triiodothyronine and the 24-hour thyroidal I^{131} uptake, the BMR, or the serum chemical PBI level in either of these groups. The PBI^{131} or conversion ratio was available in only a limited number of cases. In 1 of the 3 euthyroid subjects in whom circulating triiodothyronine was demonstrated (the patient who formerly had been thyrotoxic) the PBI^{131} level was definitely elevated. In the thyrotoxic group, PBI^{131} values were available in 14 of the 28 patients; frequently the presence of triiodothyronine was associated with a relatively high PBI^{131} value, but no consistent correlation was apparent between the PBI^{131} levels and the presence or absence of triiodothyronine. A possible correlation between the clinical status of the hyperthyroid patient and the presence of triiodothyronine was also scrutinized. In all of the cases of thyrotoxicosis, the patients had goiters that were palpably diffuse, and none had severe ophthalmopathy. We were unable to correlate clinical severity of thyrotoxicosis with the presence of radiotriiodothyronine.

The failure to demonstrate circulating acetic-acid analogues of thyroxine and triiodothyronine is not too surprising in view of the fact that they have heretofore only been demonstrated as metabolites of their parent substances in tissue systems. If these deaminated metabolites of thyroxine and triiodothyronine indeed do exist in the serum of man, the concentration may be too small to be detected by the techniques utilized. Studies are in progress to determine whether these acetic-acid derivatives can be found in serum after the intravenous administration of labeled thyroxine and triiodothyronine. Investigation of the existence of 3:3′-diiodothyronine and 3:3′:5′-triiodothyronine in the serum of man will have to await the development of more reliable techniques for the identification of these compounds.

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THE TEN-MINUTE UPTAKE OF I^{131}: A CLINICAL STUDY AND COMPARISON WITH OTHER TESTS OF THYROID FUNCTION

H. P. HIGGINS, M.D.*

The Department of Medicine, University of Toronto, and the Department of Medicine and Radioactive Isotope Laboratory, St. Michael's Hospital, Toronto, Canada

ABSTRACT

The value of estimating the ten-minute uptake of I^{131} by the thyroid was studied in 333 cases. In hyperthyroidism the deviation from normal values was greater than with the 24-hour uptake. In some mild but definite cases of hyperthyroidism, the ten-minute uptake was clearly abnormal when the 24-hour uptake was normal. The reverse did not occur. In Graves' disease the deviation from normal of the ten-minute uptake was greater than the deviation from normal of the serum protein-bound iodine level, whereas in toxic nodular goiter the degree of elevation above normal of the two values was comparable. Among 221 patients without thyroid disorders, in only 1 was the ten-minute thyroidal uptake of I^{131} above the normal range. One of 33 patients with Graves' disease, and 3 of 20 patients with toxic nodular goiter had ten-minute uptakes in the normal range. The short duration of the test renders it practicable, and allows its adaptation to the use of I^{132}. It does not distinguish iodine deficiency from hyperthyroidism; it is useless in the diagnosis of hypothyroidism, and suffers the slight disadvantage of requiring an intravenous injection.

TO BE worthy of a report in the literature, any new test of thyroid function must either demonstrate a different phase of the metabolism of iodine, increase the diagnostic accuracy of existing tests, or make possible the use of newer isotopes of iodine. A test which fufills all these requirements and is technically easy, is the subject of this report.

Many tests of thyroid function have been developed which utilize tracer doses of I^{131}. The thyroidal uptake of I^{131} may be measured at different intervals (1–3), or the thyroidal clearance of I^{131} may be measured (4–6). The urinary excretion of I^{131} may provide an indirect measurement of the concentrating capacity of the thyroid (7), and the concentration of serum protein-bound I^{131} may furnish an estimate of the rate of hormone production (8). The comparative diagnostic accuracy of many of these tests has recently been studied by McConahey *et al.* (9).

It is well known that the iodine "trapping" or concentrating function of

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* F.R.C.P. (C).

the thyroid gland, as distinct from its "protein binding" or hormone production, is a factor in producing a high concentration of iodine in the gland. This phase of iodine metabolism is not accurately reflected in practical tests of thyroid function, such as the 24-hour uptake in the neck and the 24-hour urinary excretion of I^{131}.

By the continuous registration of counts obtained with a scintillation counter over the neck during the first few minutes after an intravenous injection of I^{131}, Larsson and Jonsson were able to demonstrate the special value of a curve obtained by this method in the assessment of thyroid function, as compared with other tests (10). In the 19 cases studied they found that this curve yielded a better spread between hyperthyroid, euthyroid, and hypothyroid patients than did the 24-hour uptake and (or) the 24-hour urinary excretion of I^{131}. They also suggested that it might have special value in the investigation of out-patients because of its short duration, and noted that it reflected the iodine-concentrating capacity of the gland even when the "organic" binding of iodine was impaired.

This report demonstrates the value of such a curve in the assessment of thyroid function as compared to other tests in 333 cases.

PRELIMINARY STUDIES[1]

In studying the thyroidal clearance of I^{131} and I^{132} with counters over the neck and over the thigh and continuously recording rate-meters, it became apparent that in hyperthyroidism a graph of the early uptake of radioactive iodine does not assume the shape of a simple exponential curve. There is a very rapid uptake during the first few minutes. The curve then assumes a simple exponential form, as depicted in Figure 1B. This shows the counts minus background registered over the neck and the thigh, and plotted on semilogarithmic graph paper. In the normal subject, on the other hand, the uptake curve assumes a simple exponential form once mixing is complete (Fig. 1A).

It was possible to show that this initial curve in hyperthyroidism was due to "iodine trapping," in that it was not appreciably changed by administration of carbimazole (Neomercazole) but was blocked by 400 mg. of potassium perchlorate given one hour previously (Fig. 1, C and D). Any test which takes into account only the first few minutes of the uptake of I^{131} would reflect the iodine-concentrating or "trapping" mechanism in hyperthyroidism better than "uptakes" measured at later times.

Because of their favorable results, and our own observations concerning the nature of the early uptake of radioactive iodine, it was decided to give the test described by Larsson and Jonsson a further clinical trial (10).

[1] Work conducted under the direction of Professor Russell Fraser at the British Post Graduate Medical School, London, U.K., while holding a McLaughlin Foundation Travelling Fellowship.

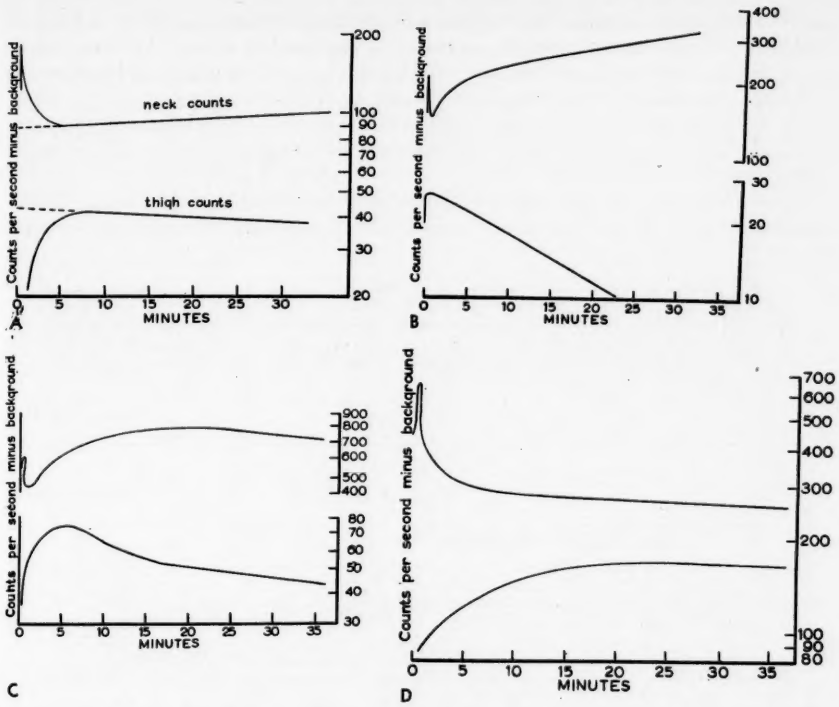

FIG. 1. Neck and thigh counts of radioactivity after intravenous injection of I^{131}: **A.** In a normal subject (euthyroid). **B.** In a case of thyrotoxicosis. **C.** In a case of thyrotoxicosis after 1 week of treatment with Neomercazole (20 mg. three times daily). **D.** In a case of thyrotoxicosis after treatment with 400 mg. of $KClO_4$.

CLINICAL MATERIAL AND METHODS

Patients

Tracer doses of I^{131} were given to: 1) euthyroid control subjects; 2) patients with anxiety states in whom the diagnosis of hyperthyroidism needed exclusion, but in whom careful clinical evaluation and follow-up led to a diagnosis of simple anxiety; 3) patients with nontoxic goiter (adenomatous goiter without hyperthyroidism); 4) patients with Graves' disease (diffuse toxic goiter) with a variable degree of exophthalmos; 5) patients with toxic nodular goiter; and 6) patients with hypothyroidism.

The final diagnosis of hyperthyroidism, euthyroidism or hypothyroidism was based on clinical evaluation, clinical course, response to treatment, and in many cases serum protein-bound iodine (PBI) concentration. It was entirely independent of radioactive iodine tests.

Methods

A tracer dose of 4 to 10 microcuries (μc.) of I^{131} was given intravenously in all cases except in the preliminary studies described. The radioactivity count over the neck was recorded continuously with a scintillation counter at a distance of 15 cm. from the esti-

mated center of the thyroid. The continuously recording rate-meter used was a Francis-Bell medical spectrometer, with its power supply stabilized by means of a Sorsen regulator, and an Esterline Angus recorder. The energy level dial was adjusted to give the maximum counts for I^{131} and the channel width was reduced to 50 pulse height units. With this equipment, the total count registered by a source of I^{131} measured at 33 cm. from the crystal was reduced by 10 per cent when the source was immersed in a beaker containing 800 cc. of water.

Curves for normal and hyperthyroid patients are shown in Figure 2, A and B. Extrapolating the curve to zero time and subtracting the result from the count over the neck

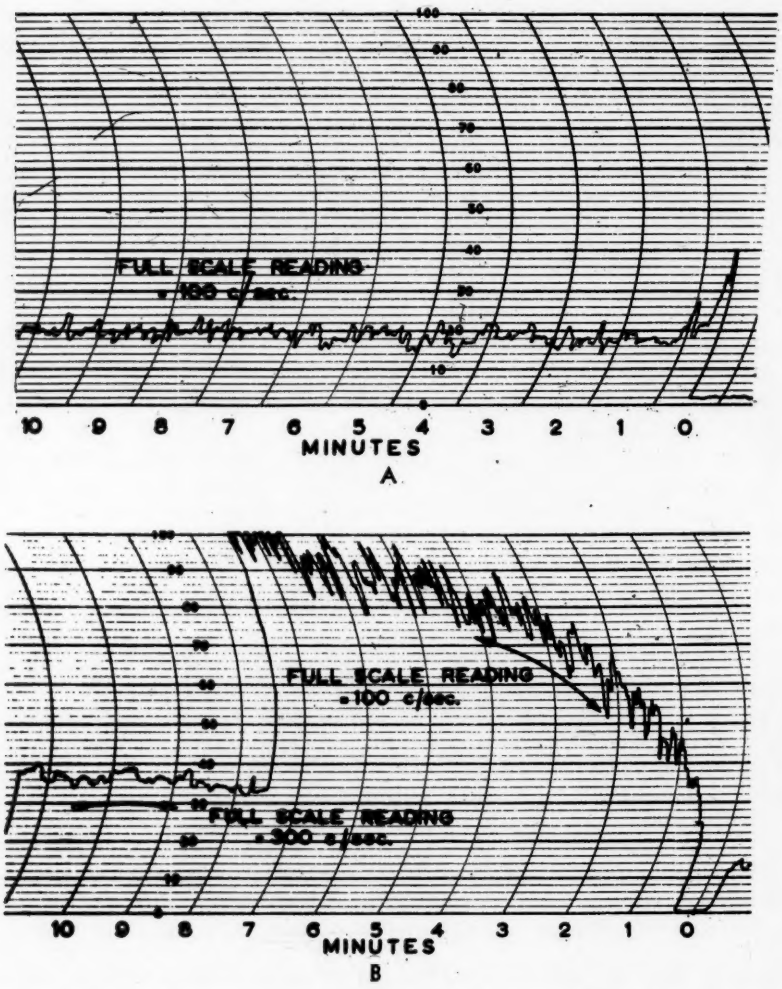

FIG. 2. Continuous records of counts over the neck after intravenous injection of 10 microcuries of I^{131}: **A.** In a normal (euthyroid) subject. **B.** In a case of thyrotoxicosis.

TABLE 1. TEN-MINUTE AND 24-HOUR THYROIDAL I^{131} UPTAKES, BY CLINICAL DIAGNOSIS AND SEX

Clinical diagnosis	Sex	No. of patients in group	10-Minute uptake (% dose)			24-Hour uptake (% dose)		
			Mean	Standard deviation	Observed range	Mean	Standard deviation	Observed range
Normal	M	8	0.61	0.52	0 to 1.2	24.5	9.9	15.6 to 39.7
	F	31	0.94	1.06	0 to 4.5	23.6	7.1	14.2 to 45.2
	T	39	0.87	0.97	0 to 4.5	23.8	7.6	14.2 to 45.2
Anxiety states	M	49	0.81	0.73	0 to 2.5	22.9	6.4	15.0 to 42.0
	F	133	0.88	0.72	0 to 2.9	24.5	6.8	14.0 to 42.2
	T	182	0.85	0.72	0 to 2.9	24.1	6.7	14.0 to 42.2
Nontoxic goiter	M	8	1.92	1.33	0 to 3.7	27.3	9.0	17.3 to 44.4
	F	36	1.86	1.94	0 to 10.0	28.4	9.1	15.0 to 44.4
	T	44	1.95	1.83	0 to 10.0	28.2	9.0	15.0 to 44.4
Graves' disease	M	7	20.4	12.8	9.4 to 47.0	54.1	13.9	38.0 to 71.8
	F	26	13.6	8.2	1.2 to 28.0	66.1	13.2	28.0 to 86.6
	T	33	15.0	9.6	1.2 to 47.0	63.7	14.0	38.0 to 86.6
Toxic nodular goiter	M	5	17.3	10.7	3.5 to 31.0	70.0	12.1	56.6 to 83.0
	F	15	8.2	6.4	1.2 to 23.5	56.4	14.4	34.6 to 89.0
	T	20	10.5	8.4	1.2 to 31.0	59.8	14.8	34.6 to 89.0
Hypothyroidism	M	4	1.62	2.32	0 to 5.2	6.2	5.2	1.5 to 12.0
	F	11	0.58	0.56	0 to 1.4	7.5	4.8	0.0 to 13.3
	T	15	0.86	1.27	0 to 5.0	7.1	4.8	0.0 to 13.3

TABLE 1A. TEN-MINUTE AND 24-HOUR THYROIDAL I^{131} UPTAKES AND SERUM PBI LEVELS, BY CLINICAL DIAGNOSIS

Clinical diagnosis	10-Minute uptake (% dose)			24-Hour uptake (% dose)			Serum PBI (μg./100 ml.)		
	No. in group	Mean	Standard deviation	No. in group	Mean	Standard deviation	No. in group	Mean	Standard deviation
No abnormal thyroid condition	221	0.86	0.77	221	24.0	6.8	39	5.0	0.81
Nontoxic goiter	44	1.95	1.83	44	28.2	9.0	10	5.9	1.6
Hyperthyroidism	53	13.3	9.2	53	62.1	14.2	28	11.7	3.0
Hypothyroidism	15	0.86	1.27	15	7.1	4.8	12	1.8	1.0

TABLE 1B. TEN-MINUTE AND 24-HOUR THYROIDAL I^{131} UPTAKES AND SERUM PBI LEVELS IN ALL CASES IN WHICH THE 3 READINGS WERE AVAILABLE

Clinical diagnosis	No. in group	10-Minute uptake (% dose)		24-Hour uptake (% dose)		Serum PBI (μg.=100 ml.)	
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Normal	39	0.87	0.97	23.8	7.6	5.0	0.81
Nontoxic goiter	10	3.17	2.74	32.3	10.6	5.9	1.6
Graves' disease	14	13.52	7.83	60.9	18.5	12.3	3.3
Toxic nodular goiter	14	7.99	6.92	54.5	11.7	11.1	2.7
Hypothyroidism	5	1.32	2.14	6.9	4.8	2.3	1.3

TABLE 2. THE 95 PER CENT CONFIDENCE INTERVALS BY DIAGNOSIS GROUPS

Clinical diagnosis	No. in group	10-Minute I^{131} uptake (% dose)	24-Hour I^{131} uptake (% dose)
No abnormal thyroid condition	233	0 to 0.96	23.2 to 24.9
Nontoxic goiter	44	1.40 to 2.50	25.5 to 30.9
Graves' disease	33	11.7 to 18.4	58.7 to 68.4
Toxic nodular goiter	20	6.5 to 14.5	52.6 to 66.9
Hypothyroidism	15	0 to 1.59	4.37 to 9.87

at ten minutes, provides a fair index of thyroidal uptake at ten minutes. This can then be expressed as a percentage of the administered dose measured in air, at the same distance from the counter.

Previous experience in carrying out thyroid clearance procedures with I^{132} and a varying volume of material indicated that the one-minute count could not be considered approximately equal to the theoretical "neck count at 0 time, mixing being complete" as suggested by Larsson and Jonsson (10). It frequently fell on the downward slope of the injection peak when larger volumes were used, or when the speed of injection was slow. Extrapolating the curve back to zero time with the aid of a transparent plastic rule, though subject to some subjective error, was independent of the rate of injection.

The use of the Francis-Bell spectrometer practically eliminates error due to backscatter radiation, and leaves only the error due to absorption of radiation by the tissues of the neck. This tends to make the neck measurements less than they should be (11, 12).

The 24-hour uptake of radioactive iodine was measured in the same patients, with the same instruments, at a distance of 33 cm. from the estimated center of the thyroid, and was corrected by subtracting the count obtained over the thigh at the same distance as for the count obtained over the neck. It was expressed as a percentage of the administered dose, measured in air at the same distance from the counter.

RESULTS

In Table 1, results of the radioactive iodine tests in the normal controls and the patients with anxiety states can be compared. Because there was no significant difference between the means of these two groups ($P > 0.9$), and because the patients with anxiety states were considered to be free of

TABLE 3. COEFFICIENT OF CORRELATION BETWEEN 10-MINUTE AND 24-HOUR THYROIDAL I^{131} UPTAKE READINGS, BY CLINICAL DIAGNOSIS AND SEX

Clinical diagnosis	Males	Females	Total
Normal	0.566	0.261	0.285
Anxiety states	0.510	0.335	0.380
Nontoxic goiter	0.691	0.333	0.384
Graves' disease	0.410	0.542	0.128
Toxic nodular goiter	0.201	0.574	0.555
Hypothyroidism	0.448	0.006	0.197

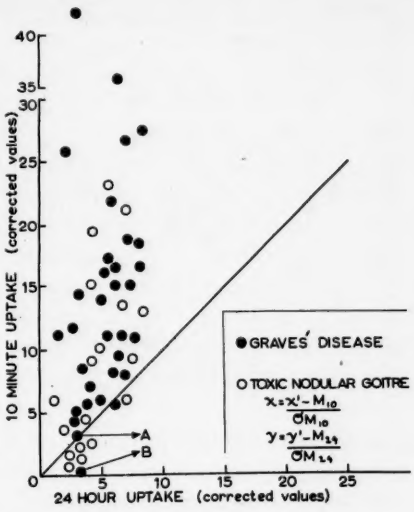

FIG. 3. Cases of Graves' disease and toxic nodular goiter plotted according to the ten-minute and 24-hour thyroidal uptakes of I^{131}. The significance of the elevation above the normal mean for each test is compared by subtracting the mean of the normal group for that test and dividing by the standard deviation of the normal group.

The higher above the mean each test lies, in terms of standard deviations, the more significant is its elevation. The line drawn at 45° from the origin represents points that are equally elevated above the mean, in terms of these "standard deviation units."

A and B represent 2 patients with Graves' disease whose chief complaints were exophthalmos, but who had mild symptoms of hyperthyroidism which responded to antithyroid drugs. They came very close to being euthyroid with exophthalmos.

any thyroid disease, the data were combined for the purpose of these studies (see Table 1A). That there was no bias shown in selecting patients for PBI tests is shown by comparing the mean of the tests in the patients of Table 1B with the mean of the tests in all the patients of Table 1A, ($P > 0.5$).

The 95 per cent confidence interval for the true mean of the group from which these samples were drawn is shown in Table 2 according to diagnosis. The ten-minute uptake is seen to be of no value in the diagnosis of hypothyroidism.

The coefficients of correlation between the ten-minute uptake and 24-hour uptake are seen in Table 3. There is a slightly positive correlation, but the coefficients are low.

The diagnostic accuracy of the tests in the diagnosis of hyperthyroidism

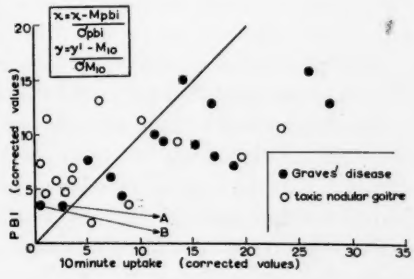

FIG. 4. Cases of Graves' disease and toxic nodular goiter plotted according to the ten-minute thyroidal uptake of I^{131} and the serum PBI concentration. The significance of the elevation is compared by plotting the result of the test in terms of the number of standard deviations above the normal mean. The line drawn at 45° from the origin represents points that are equally elevated above the mean in terms of these "standard deviation units."

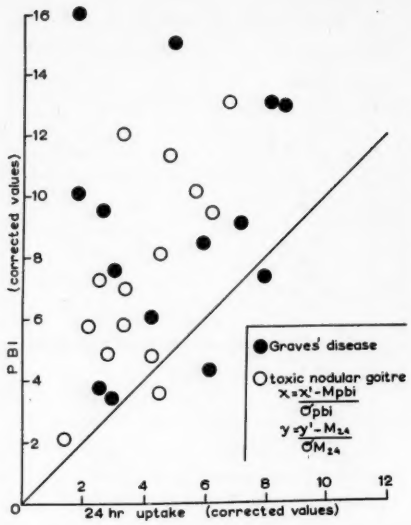

FIG. 5. Cases of Graves' disease and toxic nodular goiter plotted according to the 24-hour thyroidal uptake of I^{131} and the serum PBI concentration. The significance of the elevation is compared by plotting the result of the test in terms of the number of standard deviations above the normal mean. The line drawn at 45° from the origin represents points that are equally elevated above the mean in terms of these "standard deviation units."

is shown in Figures 3, 4 and 5. The co-ordinates of the points on the graphs are the differences between the individual values and the normal mean, expressed in terms of the standard deviation.

The line drawn at 45° from the origin represents values equally above the mean normal value for both tests.

In Figure 3 it may be seen that in most of the patients with hyperthyroidism the ten-minute uptake reached a higher level above the normal mean than did the 24-hour uptake.

As shown in Figure 4, the Graves' disease patients had ten-minute uptakes that reached a higher level above the normal mean than did the PBI values, but in the patients with toxic nodular goiter the PBI values reached a relatively higher level above the normal mean.

In Figure 5 it can be seen that the PBI values reached a relatively higher level above the normal mean in both types of hyperthyroidism, than did the 24-hour uptakes.

DISCUSSION

Though useless in the diagnosis of hypothyroidism, the ten-minute uptake exceeds the 24-hour uptake in accuracy of diagnosis of hyperthyroid states by a considerable margin. In some cases the ten-minute uptake in hyperthyroidism may be equal to the normal 24-hour uptake. As demonstrated, a great proportion of this early uptake is due to the "iodine trapping" mechanism. In Graves' disease, as compared with toxic nodular goiter, the relatively higher ten-minute uptake could result from the increased vascularity as much as from an increased ability to trap iodine.

The ten-minute uptake does not suffer in accuracy as a result of a rapid discharge rate from the thyroid. Three of the patients with Graves' disease had very high ten-minute uptakes, though their 24-hour uptakes were within the generally accepted normal range, *i.e.*, 40 per cent or less.

On the other hand, only 1 patient with Graves' disease had a ten-minute uptake in the normal range and a 24-hour uptake that was 44 per cent. This patient had a serum PBI level of 8.1 gamma per 100 ml. and only slight hyperthyroid symptoms, which responded to treatment with methimazole. Her main complaint was exophthalmos.

Of a total of 221 patients with no abnormal thyroid condition, only 1 had a ten-minute uptake above the normal range (*i.e.*, higher than 2 standard deviations above the mean of this group). One of 33 patients with Graves' disease, and 3 of 20 patients with toxic nodular goiter had ten-minute uptakes within the normal range.

The ten-minute uptake test suffers from the disadvantage of requiring an intravenous injection, but its short duration makes it practicable, and it can be conveniently combined with the 24-hour uptake and other I^{131} tests.

In the diagnosis of hyperthyroidism its accuracy compares favorably with that of the serum PBI level determined on the same patients in a first class laboratory (13). Though not supplanting the PBI determination in centers where it is impossible to set up the technically difficult chemical procedures of the PBI test, the ten-minute thyroidal uptake would increase the accuracy of I^{131} tests to a very useful degree.

The short duration of the test makes it ideally suited for use with I^{132}. Results obtained with this isotope could be compared directly with results obtained with I^{131}, by applying a correction for decay.

A continuous graphic record of the test is obtained and can be kept. Errors can be detected that might be missed by intermittent counting with a scaler.

Even in hypothyroidism, a condition in which it is useless as a diagnostic test, the ten-minute uptake could be of value in the study of thyroid function. This was evident in 1 case of hypothyroidism due to fibrosing thyroiditis (biopsy), with a serum PBI level of 1.6 gamma per 100 ml., a 24-hour thyroidal I^{131} uptake of 9 per cent and a ten-minute uptake of 5 per cent. The ten-minute uptake indicated a very rapid turnover of I^{131} by the gland, not suggested by the 24-hour uptake.

The test does not differentiate between the rapid uptake of radioactive iodine due to iodine deficiency, and that due to hyperthyroidism. One patient with a large diffuse goiter had a ten-minute uptake of 10 per cent and a 24-hour uptake of 32 per cent. The serum PBI level was 8.2 gamma per 100 ml. However, she was clinically hypothyroid, and when she was given 3 grains of thyroid daily for three months, her goiter disappeared.

The presence or absence of cardiac decompensation with edema does not

influence the diagnostic accuracy of the test, but further study is required to show whether or not cardiac decompensation influences the shape of the curve.

Acknowledgments

The author is indebted to Miss Norma Huber, R.N. for the determinations of the protein-bound iodine; to Dr. A. H. Sellars and Miss J. G. Sloman, B.A., of the Division of Medical Statistics of the Department of Public Health of the Province of Ontario for the statistical analysis of the results; and to Mr. Arthur Smialowski, of the Department of Photography of St. Michael's Hospital for the preparation of the figures and graphs.

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THE EFFECTS OF VARYING AMOUNTS OF STABLE IODIDE ON THE FUNCTION OF THE HUMAN THYROID*

WALTER D. FEINBERG, M.D., DAVID L. HOFFMAN, M.D. AND CHARLES A. OWEN, JR., M.D.

Mayo Clinic and Mayo Foundation, Rochester, Minnesota

ABSTRACT

The effect of addition of varying amounts of stable iodine to doses of radioiodine was studied in 64 euthyroid persons and in 46 with disturbed thyroid function. The addition of 1 to 2 mg. of stable iodine, as potassium iodide, reduced the uptake of radioiodine to a significantly greater degree in patients with Graves' disease than in euthyroid patients. The difference in response to the added load of iodine suggests that a relatively brief and precise test of thyroid function, an "iodide inhibition test," may be evolved from these studies. The concentration of serum inorganic iodide was found, by indirect means, to be considerably less than 0.5 μg. per 100 ml. in most instances, regardless of thyroid function. Calculation of the rate of accumulation of iodine by the thyroid revealed that, when the serum iodide level was raised to about 6 μg. per 100 ml., the normally functioning thyroid gland accepted the load and accumulated more than ten times the normal amount of stable iodide per unit time. In contrast, the hyperactive gland was capable of only limited response to such an added load. The elevated concentration of iodide in the serum after administration of iodide resulted in the cessation of secretion of labeled hormone in thyrotoxic patients, but little change occurred in euthyroid patients.

SOON after radioiodide was introduced to the study of function of the human thyroid, it was recognized that stable or carrier iodide interfered with the uptake of radioiodide by the thyroid (1). Interest in this inhibitory effect was revived in 1950 by Childs and co-workers (2) who demonstrated, in 5 patients with untreated Graves' disease, that the addition of 1000 μg. or more of stable iodide (I^{127}) to a radioiodide (I^{131}) tracer reduced the thyroidal uptake of radioiodide to normal proportions within one day. This finding, and the clinical response of Graves' disease to Lugol's solution, suggested a possible method to distinguish Graves' disease from other thyroid states.

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* Abridgment of portion of thesis submitted by Dr. Feinberg to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

METHODS

Effect of varying amounts of I^{127} on thyroidal I^{131} uptake

Iodide inhibition test. The technic for determination of *in vivo* thyroidal uptake previously described by Owen and associates (3) was used in the following manner to study a series of patients with varying degrees of thyroid function, excepting hypothyroidism: After a preliminary measurement of thyroidal uptake by means of carrier-free radioiodide,[1] the patient was given a tracer dose of radioiodide to which a measured amount of potassium iodide solution (representing I^{127}) was added. The uptake values were then compared with each other and with the clinical diagnoses.

The 110 patients so studied were divided into 3 groups: 1) those having untreated exophthalmic goiter (Graves' disease); 2) those with miscellaneous disorders, including previously treated thyroid conditions, thyroiditis, cancer of the thyroid, hyperophthalmopathic Graves' disease without hyperthyroidism, and metabolic disorders, such as pituitary disease, which might influence thyroid function; and 3) euthyroid patients, including those with other thyroid states exclusive of myxedema.

Triiodothyronine inhibition test. This test was performed by the method of Werner and Spooner (4) as modified by McConahey and Owen (5). A preliminary tracer dose of I^{131} was followed by 50 μg. of *l*-triiodothyronine given by mouth daily for four to seven days. The results of a second tracer dose of I^{131} were compared with those of the first dose. A depression of uptake of I^{131} greater than 50 per cent was interpreted as a normal response.

Metabolism of iodine before and after an iodide load

By observing the radioactivity in the blood and urine and over the thyroid, certain aspects of iodine metabolism were studied before and after stable iodide had been added to the radioiodide tracer. Carrier-free tracers were administered intravenously, and tracers with added carrier were administered orally to patients in the postabsorptive state. Concentrations of inorganic I^{131} and protein-bound I^{131} in the serum were determined in a well-type scintillation counter by methods previously described (3). Corrections were made for background, physical decay, volume of sample counted, and for the amount of inorganic I^{131} carried down with the protein precipitate (about 5 per cent). No correction was made for the small fraction of organically bound I^{131} appearing in the urine.

Values for serum protein-bound I^{127} and urinary I^{127} were determined by an adaptation of the method of Barker (6).

The rate of disappearance of iodide from the iodide space was determined from urinary excretion of I^{131} after the method of Keating and associates (7), and from serum inorganic I^{131} after that of McConahey and associates (8). Renal excretion rates and thyroidal accumulation rates were calculated by the methods of Keating and co-workers (7, 9). Iodide space was determined by the dilution technic as suggested by Keating and Albert (10).

Serum inorganic iodide was calculated by the method proposed by Stanley (11), which depends on the following relationship:

$$\frac{\text{serum } I^{127}}{\text{serum } I^{131}} = \frac{\text{urine } I^{127}}{\text{urine } I^{131}}.$$

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[1] The radioisotopes used in the study reported in this paper were supplied by the United States Atomic Energy Commission.

RESULTS

Effects of varying amounts of I^{127} on thyroidal I^{131} uptake

The effect of stable iodide (I^{127}) on the thyroidal uptake of I^{131} was studied in 110 patients. The results obtained in 64 euthyroid patients and in 23 patients with untreated Graves' disease are shown in Figures 1 and 2 and Table 1. Also included are the results obtained in 10 euthyroid patients and 5 patients with untreated Graves' disease who received carrier-free tracers on two consecutive days. It should be noted that when no carrier iodide was added, the mean difference between paired determinations was only 3 percentage points or less.

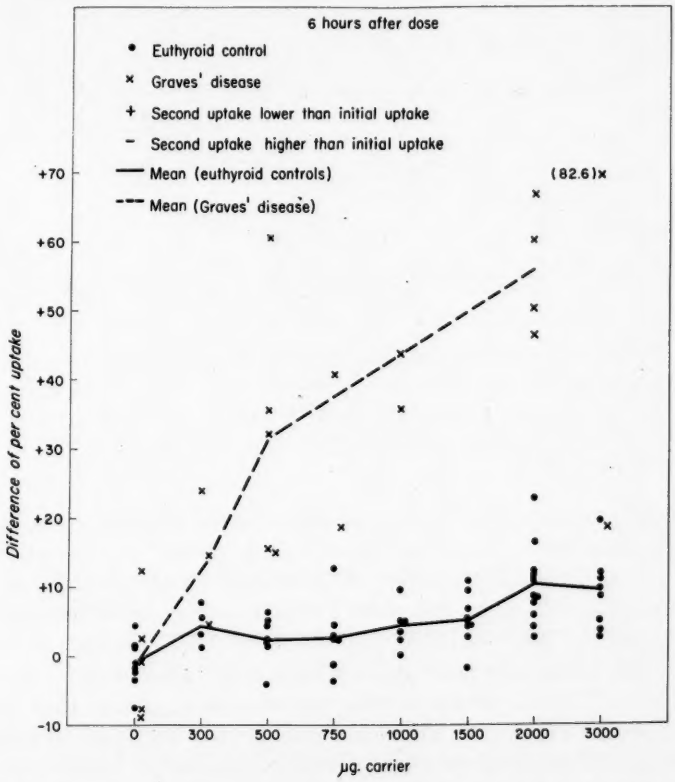

FIG. 1. Effects of varying amounts of stable iodide on the 6-hour thyroidal uptake of I^{131}. The 6-hour uptakes, calculated as a percentage of the administered dose, are compared before and after the addition of potassium iodide (expressed as micrograms of iodide). The second uptake (after added carrier) was subtracted from the first uptake (carrier-free) of each pair.

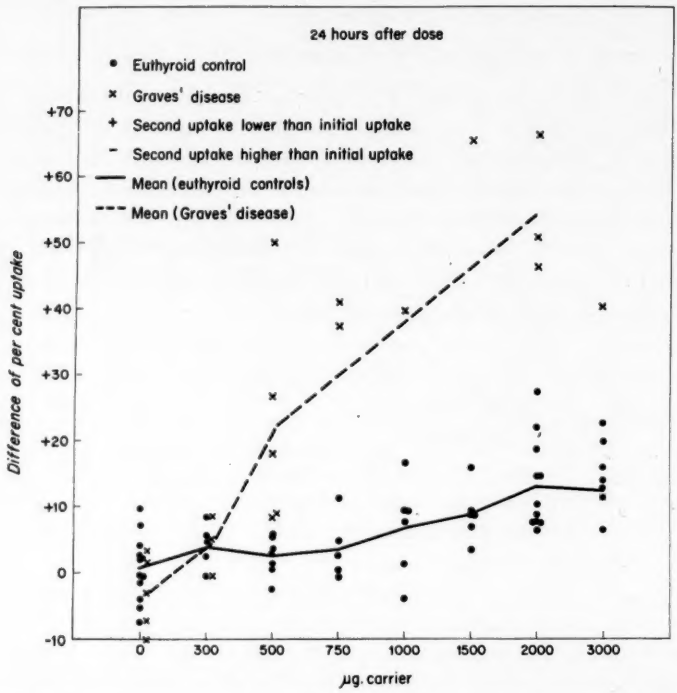

FIG. 2. Effects of varying amounts of stable iodide on the 24-hour thyroidal uptake of I^{131}. The 24-hour uptakes, calculated as a percentage of the administered dose, are compared before and after the addition of potassium iodide (expressed as micrograms of iodide). The second uptake (after added carrier) was subtracted from the first uptake (carrier-free) of each pair.

Only slight reductions of uptake of I^{131} occurred in the euthyroid group unless the amount of added iodide was 2 to 3 mg. In the group with Graves' disease, significant inhibition of uptake (more than 50 per cent) resulted consistently when less than 2 mg. of carrier iodide was added.

To express the degree of inhibition of uptake of I^{131} effected by the addition of stable iodide, the percentage uptake of I^{131} after addition of carrier was subtracted from the precarrier value. It is apparent that with an initial uptake of 30 per cent, a normal thyroid can be inhibited to a maximal extent of only 30 percentage points. However, the thyroid gland in Graves' disease, with an uptake of 65 per cent theoretically, can be depressed to a maximum of 65 percentage points.

An alternative method of expressing the results is as follows:

$$\frac{\text{initial uptake minus final uptake}}{\text{initial uptake}}.$$

TABLE 1. COMPARISON OF 6-HOUR AND 24-HOUR THYROIDAL UPTAKE OF I^{131} BEFORE AND AFTER THE ADDITION OF POTASSIUM IODIDE*

Carrier iodide (μg.)	Controls (euthyroid patients)						Graves' disease					
	No. of patients	Hours after dose	Mean uptake before add'n of carrier iodide	Mean differences of % uptake after add'n of carrier iodide†	2 S.D.‡	% difference§	No. of patients	Hours after dose	Mean uptake before add'n of carrier iodide	Mean differences of % uptake after add'n of carrier iodide†	2 S.D.‡	% difference§
None	8	6	18	− 1	7	6	5	6	66	0	17	0
	12	24	26	+ 1	10	4	5	24	74	− 3	11	4
300	4	6	14	5	6	36	3	6	68	+14	19	21
	5	24	26	4	7	15	3	24	76	4	9	5
500	7	6	17	3	7	18	5	6	74	32	37	43
	7	24	27	3	6	11	5	24	76	22	34	29
750	7	6	16	3	10	19	2	6	53	30	—	57
	5	24	25	4	9	16	2	24	74	39	—	53
1000	6	6	15	4	6	27	2	6	69	40	—	58
	6	24	26	7	14	27	1	24	85	40	—	47
1500	9	6	15	5	7	33	—	6	—	—	—	—
	6	24	25	9	8	36	1	24	90	65	—	72
2000	12	6	21	10	11	48	4	6	69	56	19	81
	11	24	35	13	14	37	3	24	70	54	21	77
3000	8	6	17	9	11	53	2	6	75	50	—	67
	8	24	28	13	15	46	1	24	81	40	—	49

* The second uptake value was subtracted from the first of each pair and the differences for each group were averaged.

† Plus sign =second uptake (with added carrier) lower than first (carrier-free). Minus sign =second uptake (with added carrier) higher than first (carrier-free).

‡ Standard deviation.

§ Percentile difference $= \frac{\text{mean difference of per cent uptake}}{\text{mean uptake before added carrier iodide}} \times 100.$

This method has the advantage of rendering percentile values which are directly comparable, regardless of the initial uptake. It has the disadvantage that slight changes in cases of low-normal thyroidal uptake (including some changes inherent in the error of the method) are magnified. Thus, a change from 22 per cent before addition of carrier to 19 per cent after addition of carrier yields a percentile difference of 14. Inspection of the tables will make it clear that by either method of calculation the reduction of uptake is significantly greater in patients with Graves' disease.

In Table 2 are listed the results obtained with the iodide inhibition test and the triiodothyronine inhibition test in 7 patients with hyperophthalmopathic Graves' disease without clinical hyperthyroidism, and in Table 3 are the results in 2 patients having the same disorder, but in whom only the iodide inhibition test was performed. Of these 9 patients, 2 had a thyroidal uptake of I^{131} suggestive of hyperthyroidism; in 4 the results of the iodide inhibition test were in the hyperthyroid range; and in 6 of 7, the results of

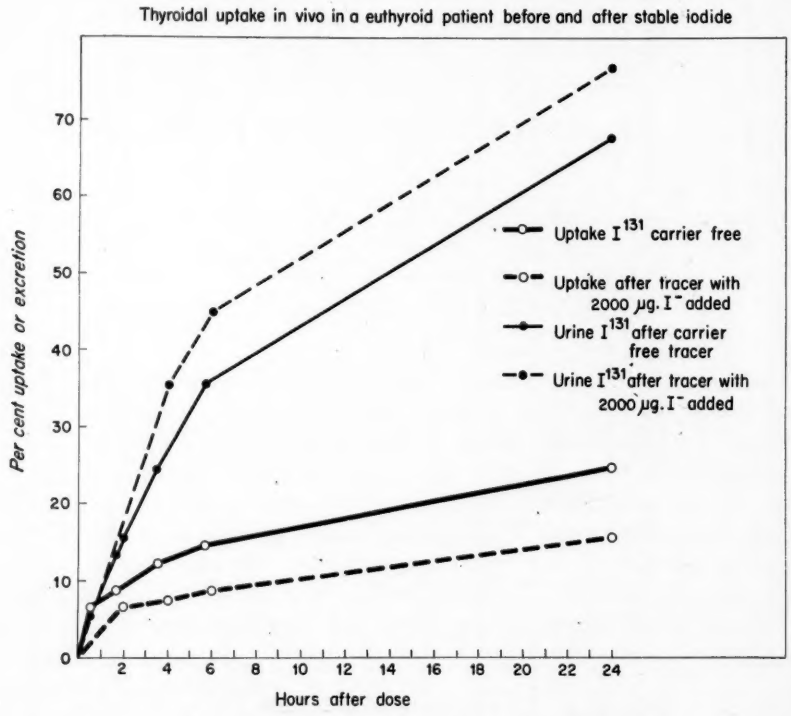

FIG. 3. Example of thyroidal uptake and renal excretion of I^{131}, before and after added carrier iodide, in a euthyroid patient.

TABLE 2. COMPARISON OF RESULTS OF IODIDE INHIBITION TEST AND TRIIODOTHYRONINE (T_3) INHIBITION TEST

Condition	Case No.	Amount of carrier iodide (μg.)	Iodide inhibition test						Triiodothyronine inhibition test			Interpretation of results	
			6-Hour thyroidal uptake (% dose)			24-Hour thyroidal uptake (% dose)			24-Hour thyroidal uptake (% dose)				
			Carrier iodide		Difference	Carrier iodide		Difference	Before	After 3–5 days T_3, 100 μg./day	Reduction	Iodide inhibition test	T_3 inhibition test
			Before	After		Before	After						
Hyperophthalmopathic Graves' disease without hyperthyroidism	1	2000	42	12	30	51	21	30	51	21	30	Abnormal	Normal
	2	2000	35	15	20	—	—	—	35	47	0	Normal	Abnormal
	3	750	25	7	18	30	10	20	30	31	0	Abnormal	Abnormal
	4	1000	27	12	15	41	22	19	41	13	28	Borderline	Normal
	5	750	23	9	14	36	19	17	36	32	4	Abnormal	Abnormal
	6	750	16	9	7	30	16	14	30	30	0	Borderline	Abnormal
	7	750	19	16	3	29	26	3	29	25	4	Normal	Abnormal
Adenomatous goiter (euthyroid)	8	750	18	6	12	23	12	11	23	4	19	Normal	Normal
Hashimoto's thyroiditis (euthyroid)	9	750	22	18	4	38	28	10	38	3	33	Normal	Normal
Euthyroid controls	10	2000	26	14	12	35	27	8	35	11	24	Normal	Normal
	11	2000	23	6	17	39	17	22	39	12	27	Borderline	Normal

TABLE 3. RESULTS OF VARYING AMOUNTS OF STABLE IODIDE ON THE THYROIDAL I^{131} UPTAKE IN PATIENTS WITH VARIOUS ENDOCRINE AND THYROID DISORDERS

Condition	Case No.	Amount of carrier iodide (μg.)	6-Hour thyroidal uptake (% dose)			24-Hour thyroidal uptake (% dose)			Interpretation of results of iodide inhibition test
			Carrier iodide		Difference*	Carrier iodide		Difference*	
			Before	After		Before	After		
Hyperophthalmopathic Graves' disease without hyperthyroidism	12	1000	43	8	+35	55	11	+44	Abnormal
	13	2000	13	11	+ 2	22	19	+ 3	Normal
Hashimoto's thyroiditis	9	750	22	18	+ 4	38	28	+10	Normal
	14	1500	62	34	+28	75	52	+23	Abnormal
Hürthle-cell carcinoma	15	1500	20	18	+ 2	30	16	+14	Normal
	16	2000	19	14	+ 5	—	—	—	Normal
Nontoxic adenomatous goiter	17	750	44	37	+ 7	60	58	+ 2	Normal
	18	750	37	27	+10	62	51	+11	Normal
Pituitary insufficiency	19	300	12	12	0	23	26	− 3	Normal
	20	500	7	9	− 2	18	14	+ 4	Normal
	21	750	8	11	− 3	16	19	− 3	Normal
	22	2000	16	11	+ 5	23	23	0	Normal
Chromophobe adenoma without hypothyroidism	23	2000	11	4	+ 7	20	6	+14	Normal
	24	2000	10	8	+ 2	17	18	− 1	Normal
Active acromegaly	25	1000	14	18	− 4	27	33	− 6	Normal
	26	1000	17	19	− 2	30	33	− 3	Normal

* Plus sign = second value (with added carrier) lower than first (carrier-free). Minus sign = second value (with added carrier) higher than first (carrier-free).

the triiodothyronine inhibition test were compatible with hyperthyroidism. In 1 euthyroid patient with Hashimoto's disease and in 1 euthyroid patient with adenomatous goiter, the results of all radioiodine tests were normal.

In Table 3 are listed the results of the iodide inhibition test in 14 patients having a variety of thyroid and metabolic disorders. The results in 13 were consistent with the clinical impressions of euthyroid states.

The thyroidal uptake curves for a euthyroid patient, made before and after addition of carrier iodide, have contours comparable to each other (Fig. 3). The thyroidal uptake curves obtained in a patient with untreated Graves' disease are shown in Figure 4. The curves for thyroidal uptake after administration of carrier iodide have a peculiar shape. Similar curves have been observed by others, both in rats (12–14) and in human beings (2, 11, 15).

Metabolism of iodine before and after an iodide load

In an attempt to explain the sharply reduced thyroidal uptake of I^{131} in thyrotoxic patients after addition of carrier iodide, 17 patients were

studied. The results of the study are recorded in Tables 4 (euthyroid), 5 (hyperthyroid), and 6 (hyperophthalmopathic Graves' disease). In all but 1 patient, the serum inorganic iodide level was found to lie between 0.10 and 0.43 μg. per 100 ml. (mean 0.28) regardless of the activity of the thyroid. One euthyroid patient had an unexplained elevation of the serum inorganic iodide level to 0.75 μg. per 100 ml. These results agree with the estimates made by Riggs (16) and by Berson and Yalow (17). The addition of 2000 μg. of carrier iodide given orally raised the values of inorganic iodide in the serum from 4.90 to 10.55 μg. per 100 ml. (mean 6.65) within the first six hours.

After an iodide load, the normal thyroid invariably increased the total amount of iodide accumulated by tenfold or more; for example, in Case 31 (Table 4), the thyroidal iodide accumulation rose from 1 to 15 μg. per hour after the ingestion of 2000 μg. of iodide. The accumulation of iodide by the thyroid glands of 3 patients with untreated Graves' disease (Cases 11,

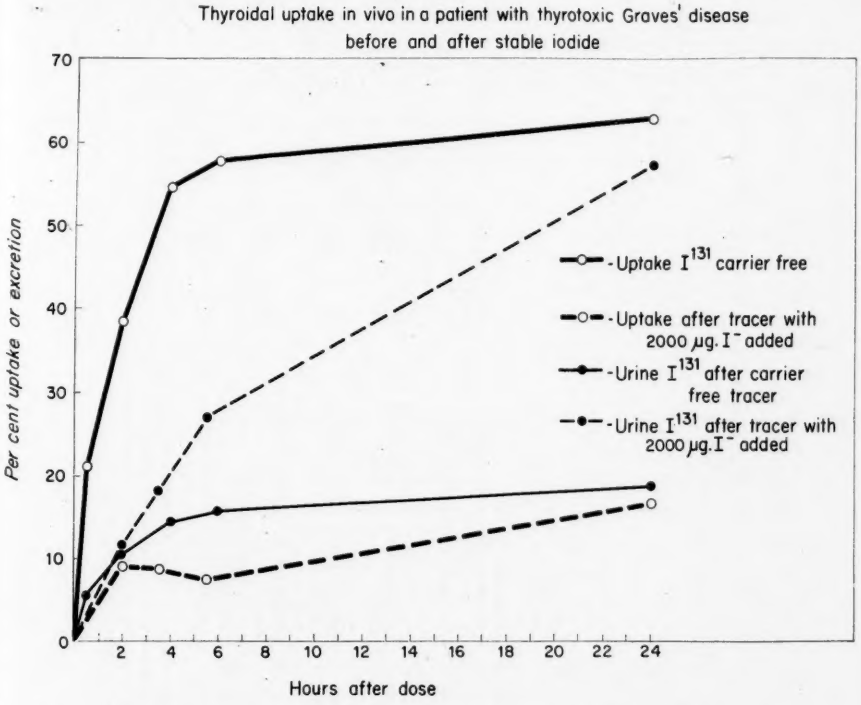

FIG. 4. Example of thyroidal uptake and renal excretion of I^{131}, before and after the addition of carrier iodide, in a patient with untreated Graves' disease.

TABLE 4. METABOLISM OF IODINE BEFORE AND AFTER AN IODIDE LOAD (EUTHYROID PATIENTS)

Case No.	Age (yrs.) & sex	Diagnosis	Carrier iodide (μg.)	24-Hour thyroidal uptake (% of dose)	Asymptotic renal excretion (fraction of dose)	Rate of disappearance (%/hr.)*	Renal excretion rate (% hr.)	Thyroidal accumulation rate (%/hr.)	Inorganic iodide in serum (μg./per 100 ml.)	Iodide space†		Thyroidal iodide accumulation (μg./hr.)	PBI^{127} in serum (μg./100 ml.)	PBI^{131} in serum after 24 hrs. (% dose /liter)
										Liters	Iodide (μg.)			
2	45 M	Idiopathic osteoporosis	Carrier-free	20	0.80	11.2	9.0	2.2	0.43	30.1	129.4	2.8	6.5	0.045
27	48 M	Nontoxic adenomatous goiter	Carrier-free	15	0.82	13.2	10.8	2.0	0.25	25.4	63.5	1.3	6.3	0.071
28	56 F	Idiopathic osteoporosis	Carrier-free 2000	29 11	0.90	4.5	4.1	0.5	10.55	14.6	1540.3	7.7	7.3	0
29	50 F	Diaphragmatic hernia	Carrier-free 2000	35 8	0.53 0.57	17.4 13.9	9.2 7.9	6.1 1.1	0.11 7.21	22.3 22.1	24.5 1607.8	1.5 17.7	6.8 6.6	0.030 0.050
30	31 F	Irritable bowel syndrome	Carrier-free 2000	24 16	0.71 0.78	18.1 13.6	12.9 10.6	4.4 2.1	0.28 5.26	16.2 19.2	45.4 852.1	2.0 17.9	7.0 7.6	0‡ 0.007
31	49 F	Exogenous obesity	Carrier-free 2000	19 11	0.68 0.75	8.0 9.2	5.4 6.9	1.5 1.0	0.29 6.37	23.5 20.8	68.2 1497.0	1.0 15.0	4.7	0.032 0‡
32	56 F	Nontoxic adenomatous goiter	Carrier-free	18	0.73	11.2	8.2	2.1	0.75	17.5	131.3	2.8	5.7	0.068

* Rate of disappearance of iodide from the iodide space as determined from serum iodide (I^{131}) levels (8).

† For calculation of the iodide space in micrograms of iodide only the initial space in liters was used, on the assumption that the data on carrier-free iodide were less subject to error than data after carrier had been added.

‡ Six hours after administration of tracer dose.

TABLE 5. METABOLISM OF IODINE BEFORE AND AFTER AN IODIDE LOAD IN PATIENTS WITH UNTREATED EXOPHTHALMIC GOITER (GRAVES' DISEASE)

Case No.	Age (yrs.) & sex	Carrier iodide (μg.)	24-Hour thyroidal uptake (% of dose)	Asymptotic renal excretion (fraction of dose)	Rate of disappearance (%/hr.)*	Renal excretion rate (%/hr.)	Thyroidal accumulation rate (%/hr.)	Inorganic iodide in serum (μg./100 ml.)	Iodide space†		Thyroidal iodide accumulation (μg./hr.)	PBI^{127} or BEI^{127} (B) in serum (μg./100 ml.)	PBI^{131} in serum (% dose /liter)	Hours after dose
									Liters	Iodide (μg.)				
11	27 F	Carrier-free	60‡	0.07	83.5	5.8	50.4	0.12	51.5	61.8	31.1	19.0(B)	0.658	6
		2000	14‡	0.86	7.0	6.0	1.0	4.90	32.9	2523.5	25.2	15.4(B)	0	6
		Lugol's§	11‖		20.8		2.4	219.3		112,939.5	2710.5	9.6(B)	0	3
33	26 F	Carrier-free	67	0.21	31.5	6.6	21.1	0.27	47.6	128.5	27.1	12.0	0.505	24
34	55 F	Carrier-free	63	0.19	27.9	5.3	17.5	0.34	33.0	112.2	19.6	11.1	0.066	6
		2000	16	0.60	12.7	7.6	2.1	6.73	18.1	2220.9	46.6	10.0	0	24
28	56 F	Carrier-free¶	55	0.40	21.0	8.4	11.6	0.33	27.2	89.8	10.4	9.3	0.003	6
35	11 F	Carrier-free¶	57	0.35	24.1	8.4	13.7	0.24	31.8	76.3	10.5	6.6	0.311	3
36	44 M	Carrier-free	62		72.2		45.1	0.35	67.1	234.9	105.9	19.3	0.118	3
37	44 M	Carrier-free¶	36		19.6		7.1	0.19	48.8	92.7	6.6	6.0	0.286	6

* Rate of disappearance of iodide from the iodide space as determined from serum iodide (I^{131}) levels (8).

† For calculation of the iodide space in micrograms of iodide only the initial space in liters was used, on the assumption that the data on carrier-free iodide were less subject to error than data after carrier had been added.

‡ Six hours after administration of tracer dose.

§ After Lugol's solution, 10 drops three times daily; last dose taken 5 hours before test.

‖ Three hours after administration of tracer dose.

¶ Case 28, Lugol's solution discontinued 4 days before test; Case 35, propylthiouracil discontinued 36 hours before test; Case 36, Lugol's solution discontinued 6 weeks before test; Case 37 (treated with I^{131} 6 months before test), propylthiouracil discontinued 7 days before test.

TABLE 6. METABOLISM OF IODINE BEFORE AND AFTER IODIDE LOAD IN PATIENTS WITH HYPEROPHTHALMOPATHIC GRAVES' DISEASE WITHOUT HYPERTHYROIDISM

Case No.	Age (yrs.) & sex	Carrier iodide (μg.)	24-Hour thyroidal uptake (% of dose)	Asymptotic renal excretion (fraction of dose)	Rate of disappearance (%/hr.)*	Renal excretion rate (%/hr.)	Thyroidal accumulation rate (%/hr.)	Inorganic iodide in serum (μg./100 ml.)	Iodide space†		Thyroidal iodide accumulation (μg./hr.)	PBI^{127} in serum (μg./100 ml.)	PBI^{131} in serum (% dose /liter)	Hours after dose
									Liters	Iodide (μg.)				
38	58 F	Carrier-free‡	46	0.40	17.3	6.9	8.0	0.22	22.5	49.5	4.0	8.4	0.140	6
39	44 F	Carrier-free	43	0.38	15.4	5.9	6.7	0.17	34.5	58.7	3.9	3.2	0.915	24
		2000	1§	0.90	9.2	8.3	0.09	6.47	21.6	2232.2	2.0		0.078	24
1	42 F	Carrier-free	51	0.39	22.5	8.8	11.4	0.10	22.3	22.3	2.5	4.6	0.026	24
		2000	21	0.44	18.1	8.0	3.7	5.01	21.7	1117.2	41.3	5.1	0.022	24
		Carrier-free‖	13		11.1		1.4	0.32		71.4	1.0		0	6

* Rate of disappearance of iodide from the iodide space as determined from serum iodide (I^{131}) levels (8).

† For calculation of the iodide space in micrograms of iodide only the initial space in liters was used, on the assumption that the data on carrier-free iodide were less subject to error than data after carrier had been added.

‡ Tapazole discontinued 3 days before test.

§ Value assumed for purposes of calculation. Actual value obtained was zero.

‖ After *dl*-triiodothyronine for 6 days (100 μg. orally daily).

33 and 34, Table 5) averaged seven times greater than normal; but after the addition of carrier iodide, the rate of increase was only about two and a half times greater in 1 patient (Case 34) and actually decreased in another (Case 11). Examination of the thyroidal uptake curve after carrier iodide was given (Fig. 4) suggests that these calculated values for accumulation of iodide in thyrotoxic patients are subject to the criticism that a true state of equilibrium probably was not approached.

Even more remarkable than the values for iodide accumulation were changes in the serum level of protein-bound I^{131} after an iodide load. In 1 euthyroid patient (Case 29, Table 4) the concentration of protein-bound I^{131} at twenty-four hours was about the same before and after the addition of 2000 μg. of iodide carrier. In 2 patients with untreated Graves' disease (Cases 11 and 34, Table 5), on the other hand, there was no demonstrable protein-bound I^{131} six and twenty-four hours after an iodide load of 2000 μg. One patient (Case 11, Table 5) was given Lugol's solution for ten days; during this time the butanol-extractable iodine of the serum diminished at the same rate as it did in normal persons, that is, about 5 per cent per day.

Renal excretion rates, and therefore renal clearance, remained relatively constant regardless of the thyroid state or of the iodide load.

COMMENT

Significant differences in the metabolism of iodine by the thyroid glands of normal persons and those with exophthalmic goiter are well recognized. The hyperactive gland accumulates more iodine per unit time and secretes more hormone than the normal gland. Whether the nature of the secreted hormone (thyroxine-triiodothyronine), its binding to the plasma proteins, or its peripheral metabolism is different in hyperthyroid and euthyroid persons is not yet clear. Another group of differences is emphasized in the following paragraphs:

First, the oral administration of iodine, such that the concentration in the plasma has increased from less than 0.5 μg. to about 6 μg. per 100 ml., results, in the euthyroid gland, in a proportional rate of uptake of iodine which is increased more than tenfold. However, in the patient with Graves' disease, only a limited increase in uptake is observed. This might be interpreted to mean that the uptake function of the normal gland is far below its maximal capability, and that the hyperthyroid gland is functioning near its capacity.

Second, since the uptake of radioiodine is presumably a direct reflection of the metabolism of the stable iodine with which it is mixed throughout the body, the observation of approximately the same percentile uptake of I^{131} before and after addition of carrier iodide, despite a tenfold increase in

the blood level of iodide, represents a tenfold increase in the quantity of stable iodide being accumulated by the thyroid gland. This was found to be the pattern in euthyroid patients when the amount of iodine added was not too taxing, that is, 1 to 2 mg.

On the other hand, administration of 2 mg. of iodine to patients with Graves' disease also increased the concentration of stable iodine in the plasma some tenfold, but in these cases thyroidal accumulation of stable iodide did not increase commensurately; instead, the percentile uptake of I^{131} was sharply reduced. This difference between normal and hyperactive thyroids seems sufficiently great to merit continued study of this phenomenon as a clinical test of possible use. VanderLaan (18) has come to the same conclusion, but with larger doses of iodide and with different timing.

Third, in 2 patients with thyrotoxicosis, the oral administration of 2 mg. of iodide, as potassium iodide, completely interrupted the appearance of I^{131}-labeled hormone in the blood. This may indicate a real difference in function between normal and overactive glands, since euthyroid persons continued to secrete labeled hormone even when accumulation of iodine per hour was increased to levels comparable to those found in Graves' disease.

As Stanley (11) observed, the amount of iodine administered is probably critically important in differentiating euthyroid and hyperthyroid function. He reported that organic binding of iodine by the thyroid was blocked in Graves' disease by concentrations of iodide of 5 μg. per 100 ml. of serum, whereas significantly higher serum levels were required in patients with less hyperplastic glands. In our studies the average concentration of iodide in the serum was about 6 μg. per 100 ml. within six hours after an oral dose of 2000 μg., regardless of the thyroidal state. Stanley (11) interpreted this interruption of secretion of hormone by the thyroid in Graves' disease on the basis of diminished synthesis of hormone resulting from an interference with the tyrosyl-iodination mechanism. Studies in rats by Wolff and Chaikoff (19) have led to the same conclusion.

The rapidity with which changes may be observed suggests, at least in Graves' disease, that the primary site of action of iodide lies within the thyroid gland itself (20–22). On the other hand, we have not followed the euthyroid patients long enough to eliminate from consideration effects on the thyroid-pituitary axis such as those suggested by Loeser (23) and by Albert (24).

If the thyroid is indeed "poisoned" by excess iodine in such a way that the synthesis of hormone is impeded, in accord with the findings of Ansell and Miller (25), the effect would seem to parallel more closely that of drugs such as thiouracil, which permit iodide uptake but not hormone synthesis. In the one patient studied during ten days' administration of

Lugol's solution, the level of butanol-extractable iodine in the serum fell steadily, and at a normal rate, warranting the conclusion that no new hormone was being added to the blood. Regardless of the precise mechanism by which iodine inhibits hormone synthesis, the pioneer clinical observations of Plummer (26) with Lugol's solution would appear to be supported.

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TREATMENT OF MALIGNANT EDEMATOUS EXOPHTHALMOS BY IMPLANTATION OF THE PITUITARY WITH YTTRIUM^{90}: REPORT OF TWO CASES

G. M. MOLINATTI, M.D., F. CAMANNI, M.D. AND A. PIZZINI, M.D.

The Institute of Medical Pathology and the Department of Endocrine-Surgery, University of Turin, Italy

ABSTRACT

Two cases of malignant exophthalmos refractory to the usual medical and roentgenologic therapy were treated by implantation of the pituitary with radioyttrium (Y^{90}). In both cases prompt and marked improvement of the ocular symptomatology occurred, in association with normalization of thyroid function. These results persisted in one case for at least sixteen months after the implantation; the other case was followed for only one month. No adrenocortical deficiency was observed. This effect of implantation of Y^{90} is interpreted as due to selective radiobiologic inhibition of the hyperactive pituitary cells which produce the thyrotropic hormone.

MALIGNANT edematous exophthalmos is generally attributed to excessive secretion of thyrotropin (TSH) or of a closely related material called "exophthalmos-producing substance" by the pituitary (1, 2). Exceptionally severe cases exist in which all forms of medical or roentgenologic treatment are unsuccessful and progressive protrusion of the eyeballs with consequent corneal and conjunctival changes lead to blindness.

Based on the concept of the pituitary etiology of malignant exophthalmos, cauterization of the pituitary with or without section of the pituitary stalk has been successfully performed in 9 cases (3, 4), and surgical total (5) or subtotal (6) hypophysectomy in 2 cases.

Recently radiologic destruction of the hypophysis by implantation of radioactive substances via the nasal trans-sphenoidal route for the palliative treatment of tumors, has been found to be much less traumatic than hypophysectomy.

Yttrium^{90} (Y^{90}) has been widely used as a pure source of beta radiation with no damaging effect on the surrounding nervous structures (7–10).

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The simplicity of this radiosurgical method, which has no significant risk, suggested to us its use in the treatment of 2 extremely severe cases of malignant exophthalmos which proved refractory to the usual medical and roentgenologic treatment.

CASE REPORTS

Case I

The patient, a 60-year-old male with an eight-month history of severe hyperthyroidism, was seen by us in March 1956. He had pronounced bilateral exophthalmos (O.D., 33 mm.; O.S., 34 mm.), which developed after x-ray treatment of the thyroid.

He was treated by roentgen irradiation of the diencephalo-pituitary region (total dose of 2100 r on two temporal fields), estrogen (Hexestrol, 5 mg. daily), desiccated thyroid (0.3 Gm. daily) and methimazole (Tapazole, 25 mg. daily). After a month, there was marked improvement of the symptoms of hyperthyroidism but only a slight reduction of the exophthalmos (right eye, 30 mm.; left eye, 31 mm.). Antithyroid treatment at a lower dosage level, plus desiccated thyroid, estrogen and pineal extract was continued for several months. Temporary discontinuation of treatment in July 1956 was followed by a prompt rise of the basal metabolic rate (BMR), a drop in the blood cholesterol level, tachycardia and loss of weight. The maintenance of a euthyroid state required the constant administration of moderate doses (100 to 200 mg.) of methylthiouracil.

Exophthalmos, however, proved to be resistant to all forms of treatment. Severe corneal and conjunctival changes, marked edema, retraction of the eyelids and extraocular muscle palsies (Fig. 1B) were also present. Implantation of the pituitary with a pellet of Y^{90} (9 mc.) was performed on April 17, 1957, and all medication was omitted.

Following the implantation the patient had moderate headache which disappeared after a week. No significant diuresis or changes in blood pressure were observed. Neither immediately after the operation nor during the following months, were there any clinical manifestations referable to adrenocortical insufficiency. The urinary excretion of 17-ketosteroids and 17-hydroxycorticoids, after an initial increase, was constantly within normal limits.

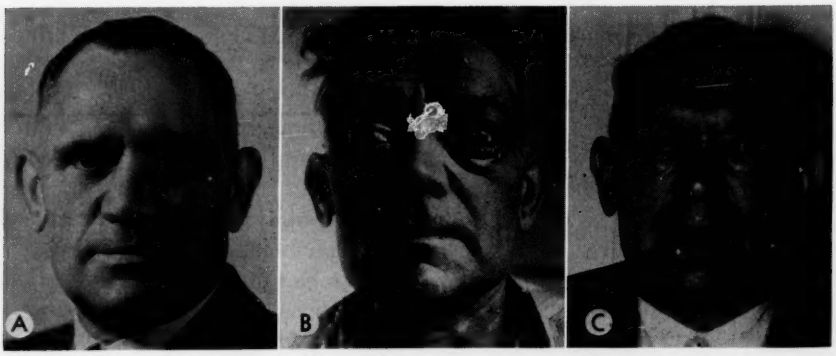

FIG. 1. *Case I:* **A)** Four years before the onset of the disease. **B)** One month before implantation of the isotope. **C)** Thirteen months after implantation of yttrium^{90} into the pituitary.

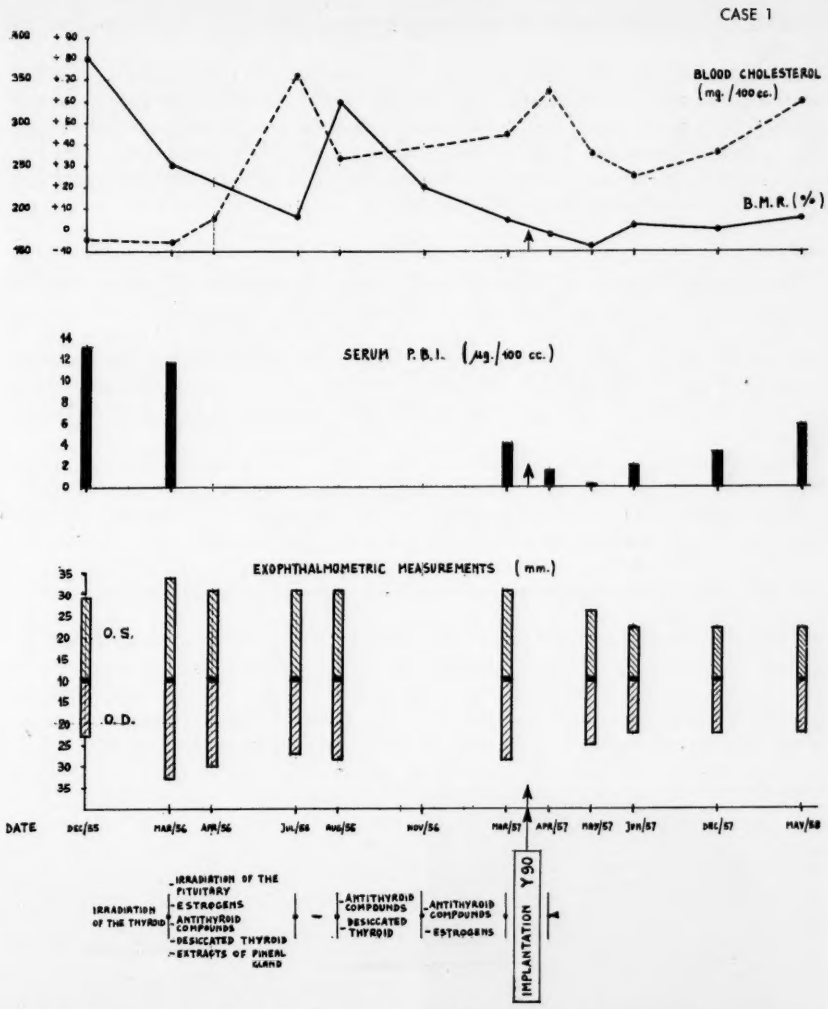

FIG. 2. *Case I:* Metabolic (BMR: serum total cholesterol and PBI levels) and exophthalmometric changes induced by various forms of medical and roentgenologic treatment and finally by implantation of yttrium^{90} into the pituitary.

Repeated determinations of the BMR and of the blood cholesterol level[1] in the 16-month period since implantation of the radioactive isotope showed normal values (Fig. 2). The serum protein-bound iodine (PBI) dropped to extremely low levels (0.2 to 0.4 gamma per 100 ml.) a few weeks after the implantation and returned to normal levels

[1] A recent increase in the concentration of blood cholesterol in this patient could be related to the evident atherosclerotic changes rather than to thyroid hypofunction.

during the following months. The value was recently found to be 5.8 gamma per 100 ml. (Fig. 2).

Prompt improvement of the exophthalmos occurred. Within two months the measurements dropped from 30 mm. for the right eye and 31 mm. for the left, to 22 mm. for each eye (Fig. 2). The cornea became bright and transparent, and the conjunctival changes, edema, and the retraction of the eyelids disappeared (Fig. 1C). At the time of writing, the exophthalmos has not diminished further. No signs referable to hyperthyroidism were present throughout this period (Fig. 2).

Case II

The patient, a 47-year-old male suffering from thyrotoxicosis since October 1954, was referred to us in February 1956 with a clinical picture of hyperthyroidism and exophthalmos (O.D., 21 mm.; O.S., 22 mm.). The level of serum PBI (11 gamma per 100 ml.) and the BMR (+44 per cent) were increased. The blood cholesterol level was subnormal (100 mg. per 100 ml.). The thyroidal I^{131} uptake was markedly increased (60 per cent after five hours; 41 per cent after twenty-four hours; 15 per cent after forty-eight hours).

Treatment with estrogen (Hexestrol, 5 mg. daily), methimazole (Tapazole, 30 mg. daily) and desiccated thyroid (0.3 Gm. daily) was started. After a few days there was evident improvement of thyroid function, but the exophthalmos remained unchanged. The antithyroid treatment (methimazole and desiccated thyroid) was continued for several months but failed to relieve all the clinical and metabolic signs of thyroid hyperfunction. A slight but progressive parallel increase of the exophthalmos was noted (O.D., 22 mm.; O.S., 24 mm.).

In October 1956 subtotal thyroidectomy was performed. This, associated with prolonged treatment with desiccated thyroid and carbimazole (Neomercazole), resulted in more marked improvement of the hyperthyroidism (BMR +5 per cent). However, temporary discontinuation of the medication was followed by the prompt reappearance of the clinical and metabolic signs of hyperthyroidism (BMR +50 per cent).

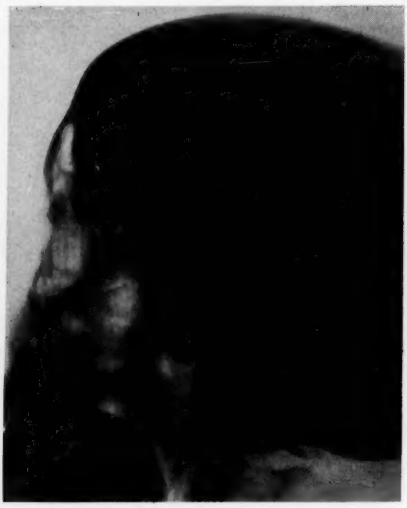

FIG. 3. *Case II:* Roentgenogram of the skull after implantation of the pituitary with two pellets of $yttrium^{90}$.

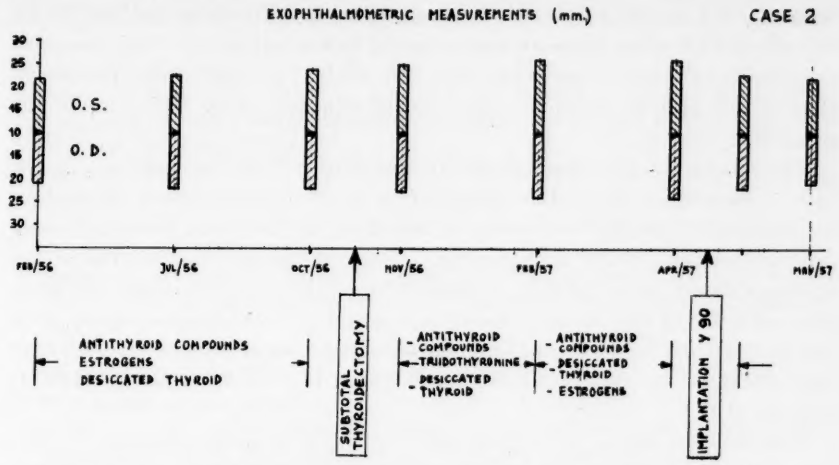

FIG. 4. *Case II:* Exophthalmometric measurements before and after implantation of the pituitary with yttrium^{90}.

Simultaneously the exophthalmos increased to a pronounced degree. The measurements reached 24 mm. for the right eye and 26 mm. for the left. Intense congestion of the conjunctiva and corneal ulcers appeared. In view of the inability to control the hyperthyroidism and especially the severe exophthalmos, two pellets of yttrium^{90} (18 mc.) were implanted into the pituitary on April 17, 1957 (Fig. 3).

Although all forms of antithyroid treatment were discontinued, a reduction of the BMR (+24 per cent) and improvement of the clinical picture were demonstrable during the third week after the implantation. No blood pressure changes or other clinical or hormonal signs of adrenocortical insufficiency were observed.

A significant drop in exophthalmometric measurements was observed as early as the tenth day following the implantation. This improvement continued. After the first month, the values were 21 mm. for the right eye and 22 mm. for the left eye (Fig. 4). Unfortunately, no follow-up of this patient was possible.

DISCUSSION

Implantation of the pituitary with Y^{90} in these 2 men was followed by prompt normalization of thyroid function, associated with a surprising degree of improvement of the ocular symptoms which had previously proved refractory to both medical and roentgenologic therapy. The regression of exophthalmos in Case I reached its maximum two months after the implantation, and no further diminution occurred during the following months.

Immediately after implantation of the pituitary with Y^{90}, both the BMR and the serum PBI level dropped to subnormal values, but urinary steroids were not altered. In Case I, adrenocortical function remained normal at

least sixteen months after the implantation. At this time the BMR and the serum PBI concentration had returned to normal levels. These findings suggest that implantation of the pituitary with Y^{90} had inhibited the excess thyrotropic activity without significantly affecting other functions of the pituitary.

The reason for this discriminative action of Y^{90} on the pituitary is not fully understood. A possible explanation is that in malignant edematous exophthalmos treated with implantation of Y^{90} into the pituitary, only partial necrosis occurs, preferentially affecting the amphophil cells which are considered to be the site of production of thyrotropin. That implantation with Y^{90} at the dosage level usually employed causes necrosis of varying degree, but seldom leads to complete functional deficiency, has been demonstrated by our observations in patients with malignant pituitary tumors (10).

The problem arises as to why the destructive process seems to involve the cells from which the excess thyrotropin originated. In malignant exophthalmos, Albeaux-Fernet and co-workers (5) observed a pituitary histologic picture characterized by a numerical increase of amphophils with histochemical signs of increased secretory activity. These cells, on account of their functional hyperactivity, could be more radiosensitive than other cell types, according to the classic concepts of radiobiology. Under these particular conditions, selective pituitary destruction could be obtained, thus justifying the hypothesis of dissociation of other aspects of pituitary function from the regression of exophthalmos.

It is our belief that implantation of the pituitary with Y^{90}, being a relatively mild surgical intervention and almost without danger of accidents, represents a useful therapeutic weapon in the treatment of severe forms of malignant exophthalmos.

Acknowledgment

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IDIOPATHIC HYPOPARATHYROIDISM: UNUSUAL RESPONSE TO VITAMIN D

D. ROBERT AXELROD, M.D.

The Metabolic Research Unit of the Medical Service, Brooklyn Veterans Administration Hospital, Brooklyn, N. Y.

ABSTRACT

A balance study of a patient with idiopathic hypoparathyroidism demonstrated an abnormal response to vitamin D_2. Dosages up to 1 million units daily did not correct the abnormal calcium and phosphorus metabolism, but dihydrotachysterol in a dosage of 3 cc. daily reversed the metabolic effects. Six years before this study, the patient had first experienced convulsive seizures. At that time, and five years later when lenticular cataracts developed, the serum calcium concentration was normal. The diagnosis of hypoparathyroidism was made at the time the patient entered the hospital for eye surgery, when hypocalcemia and hyperphosphatemia were present. The patient also exhibited trophic changes in the fingernails and episodes of psychotic behavior. In addition to the abnormal serum calcium and phosphorus levels, there were low serum levels of citric acid, increased renal reabsorption of phosphate, and a normal response to intravenous parathyroid hormone.

HYPOPARATHYROIDISM is intimately associated with abnormal calcium and phosphorus metabolism and most of the signs and symptoms of the disease have been thought to be directly related to the abnormal serum concentration of calcium. The tetany and convulsions can, in general, be correlated with acute changes in calcium concentration, but the later manifestations of the disease (lenticular cataracts, skin and nail changes, elevated cerebrospinal pressure with papilledema, mental retardation and psychotic behavior) have been correlated only with the duration of the hypocalcemic state. At the present time, the diagnosis of hypoparathyroidism cannot be made unless a low serum calcium level has been found. In the case to be presented, symptoms referable to hypocalcemia occurred six years before the diagnosis was established.

Three drugs used in the treatment of hypoparathyroidism are parathyroid extract, vitamin D_2 (calciferol) and A.T.10 (dihydrotachysterol). Each has been used effectively in combatting the disease, but the sequence of changes in calcium and phosphorus metabolism varies with the drug (1, 2). The choice of treatment in chronic hypoparathyroidism lies between

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vitamin D and A.T.10, since it is generally agreed that parathyroid extract frequently becomes ineffective after a period of time (3). There is disagreement about the relative efficacy of A.T.10 and vitamin D_2 in this condition. Many clinical reports state that A.T.10 is more effective (4, 7); others state that vitamin D_2 is just as effective as A.T.10 when doses of equal weight of active material[1] are given (5, 6). No previous report has been found in which doses of vitamin D_2 equal to, or greater than an effective dose of A.T.10, were ineffective in the initial treatment of hypoparathyroidism. In this balance study the patient failed to show any response to as much as 1 million units (25 mg.) of vitamin D_2, but did respond well to less than 4 mg. of A.T.10 per day.

CASE REPORT[2]

W.M.K., a 28-year-old Negro male, was admitted to the Ophthalmological Section of the Brooklyn Veterans Administration Hospital on March 21, 1957, with bilateral cataracts.

He had entered the U. S. Army in February 1950, and had maintained his previous good health until the early part of 1951, when cramps in his feet first developed. In June of that year he had his first convulsive seizure. This started with cramps of the legs and arms which lasted for hours, and was followed by transient dizziness and then unconsciousness. From that time, he continued to have frequent cramps of his legs and arms but could not recall when, or how frequently these were followed by unconsciousness. In November 1951, he entered an Army Hospital, where an electroencephalogram was interpreted as normal. The serum calcium level at that time was 11.9 mg. per 100 cc. In December 1951, a grand mal seizure was observed in the hospital, and treatment with Dilantin and phenobarbital was started. An electroencephalogram obtained in January 1952 revealed bitemporal spike foci and was interpreted as abnormal. His intelligence quotient measured by the Bellevue-Wechsler test was 69. In February 1952, he manifested paranoid behavior which was attributed to Dilantin toxicity; Mesantoin was substituted. He continued to have infrequent convulsive seizures and was discharged from the Army in October 1952. The diagnosis was idiopathic epilepsy. He was seen at intervals at a Veterans Administration outpatient clinic, where the anticonvulsant medication was maintained. Frequent episodes of arm and leg cramps continued, but again the patient was not able to recall the frequency of convulsions, since he has amnesia for these periods. Because of complaints of dimming vision, he was seen in May 1956 by an ophthalmologist who made a diagnosis of bilateral subcapsular cataracts. At that time the serum calcium level was 9.2 mg. and the serum phosphorus[3] level was 4.8 mg. per 100 cc. He was admitted to this hospital ten months later for surgical removal of the cataracts.

On admission to the Ophthalmological Section, both pupils had cortical "spoke-wheel" opacities. A tentative diagnosis of bilateral congenital cataracts was made. On March 26, 1957, for the first time, the serum calcium level was found to be low (5.9 mg.

[1] One cubic centimeter of A.T.10 contains 1.25 mg. of dihydrotachysterol, and a 40,000-unit dose of vitamin D_2 contains 1 mg. of calciferol.

[2] The dental aspects of this case are reported elsewhere (4).

[3] Throughout this paper the term serum phosphorus indicates serum inorganic phosphorus.

per 100 cc.), and the serum phosphorus level high (6.1 mg. per 100 cc.). A diagnosis of chronic hypoparathyroidism was made. The left lens was removed on April 4, 1957. On April 29, the patient had a grand mal seizure. There were no muscle cramps or tetany prior to, or since this seizure. He was transferred to the Metabolic Research Unit on May 1, 1957, for evaluation and treatment of the hypoparathyroidism. His past history was unremarkable except for syphilis, diagnosed in September 1951, and treated with penicillin. There was no evidence of central nervous system involvement.

On admission to the Metabolic Research Unit, examination of the patient revealed a Negro male of dull normal intelligence, and of average height and weight (71 inches and 136 pounds, respectively). There were no skeletal defects. The left eye showed the operative residua of the iridectomy, and there was a cataract of the right lens. Close to the base of the nail of each finger were pronounced trophic changes (Fig. 1). The right ear drum was scarred and perforated, and a small amount of purulent material exuded. There was a left varicocele.

Dental x-ray films showed no abnormality of the lamina dura or enamel, but did demonstrate thickening of the trabeculae and reduction of the marrow space of the mandible. X-ray examination of the chest, long bones of the extremities, hands, vertebrae, sacrum and pelvis yielded normal findings. The right mastoid area showed changes consistent with a chronic middle-ear infection. There was no evidence of intracerebral calcification. An electroencephalogram indicated a midline diencephalic disturbance, typical of a convulsive disorder. An electrocardiogram (obtained when the serum calcium level was 7.9 mg., and the inorganic phosphorus level was 6.4 mg. per 100 cc.), was normal, with a normal QT interval. Results of urinalysis, blood cell counts, and determination of the levels of serum urea nitrogen, creatinine, and glucose were all within normal limits. Serum albumin and globulin concentrations were normal—4.8 Gm. and 3.7 Gm. per cent, respectively. Serum electrolyte levels were normal—sodium 140 mEq., potassium 3.9 mEq., chloride 100 mEq., and carbon-dioxide combining power 29.6 mM. per liter. Results of liver function tests were normal except for a prothrombin time of 18.6 seconds (38 per cent of normal). Other serum levels were: alkaline phosphatase 11.0 King Armstrong units, cholesterol 292 mg., and cholesterol esters 156 mg. per 100 cc.

A phosphate reabsorption test showed that 97 per cent of the filtered phosphorus was reabsorbed by the renal tubules, and the phosphate excretion index was −.27. Creatinine clearance was normal. The response to intravenous parathyroid extract was normal, *i.e.*, the excretion of phosphorus was 7 mg. and 14 mg. per hour, and rose to 34 mg. and 29 mg. per hour after 200 units of parathyroid hormone was given intravenously.

FIG. 1. Trophic changes in the nails of a patient with idiopathic hypoparathyroidism. Photograph taken during period III of the balance study.

Metabolic study

On May 1, 1957, a fixed, analyzed diet from the metabolic kitchen was started. The patient also received 12 Gm. of supplementary calcium gluconate per day, bringing the total daily intake of calcium to 1.35 Gm. The daily phosphorus intake was 0.73 Gm. These intakes were maintained for the entire period of the balance study. Since admission to the hospital, he had been receiving a multivitamin preparation which contained 1,600 units of vitamin D_2. This was continued throughout the study, but was not included in calculating his vitamin D_2 intake. Urine was analyzed for calcium and phosphorus for each 24-hour period. Stools were analyzed for calcium, phosphorus and nitrogen, in three-day pooled collections marked by carmine red. Analyses of serum for calcium, phosphorus and citric acid were performed about three times per week. Standard analytical procedures were used for calcium, phosphorus and citric acid (7–9). Specific collection methods are reported elsewhere (4).

Collections were started on May 12, 1957 after eleven days of equilibration with the diet. Most of the data are presented on the basis of three-day periods, but some are presented on a daily basis. For the three-day periods, the daily urine values are summed; for the daily data, the feces values are divided by 3 (Table 1, Figs. 2 and 3).

After 3 control periods (nine days), the patient received 100,000 units (2.5 mg.) of vitamin D_2 by mouth daily for three periods. During periods VII and VIII, 250,000 units (8.25 mg.) of vitamin D_2 daily were given orally, and for period IX intramuscularly. The dosage of vitamin D_2 was increased to 1 million units (25 mg.) per day intra-

TABLE 1. BALANCE STUDY OF A CASE OF IDIOPATHIC HYPOPARATHYROIDISM TREATED WITH VITAMIN D_2 AND A.T.10

3-Day period	Therapy per day	Serum			Urine		Feces		Total phosphorus (Gm./period)
		Ca (mg./100 cc.)	P (mg./100 cc.)	Citric acid (mg./100 cc.)	P (mg./period)	Ca (mg./period)	P (Gm./period)	Ca (Gm./period)	
I	0	7.3	6.8	1.47	837	.05	1.40	4.01	2.24
II	0	7.3	6.8	1.71	723	.05	1.33	4.58	2.05
III	0	7.4	6.9	1.35	797	.05	1.14	3.91	1.94
IV		7.1	6.8	1.52	584	.05	1.29	3.89	1.87
V	Vitamin D, 100,000 units	6.8	5.7	1.53	488	.05	1.56	4.84	2.05
VI		7.9	7.2	1.48	890	.05	1.28	3.99	2.17
VII		6.8	7.5	—	753	.05	1.31	4.04	2.06
VIII	Vitamin D, 250,000 units	6.2	7.3	1.76	824	.05	1.20	3.96	2.02
IX		6.2	7.3	1.54	777	.05	1.30	4.28	2.08
X	Vitamin D, 1,000,000 units	6.4	7.4	1.46	804	.05	1.37	4.13	2.17
XI		6.1	7.1	1.25	669	.05	1.43	4.30	2.10
XII	A.T.10 (3 cc.)	6.2	7.3	1.59	847	.05	1.33	4.15	2.18
XIII	0	6.2	7.4	2.62	927	.05	1.30	4.01	2.23
XIV	A.T.10 (3 cc.)	6.4	7.2	1.29	1,207	.05	.95	4.43	2.16
XV	A.T.10	7.5	6.5	1.53	1,416	.05	.69	3.46	2.11
XVI	A.T.10+parathy. extr.	7.7	6.3	1.60	1,228	.05	.67	3.00	1.90
XVII	A.T.10 (3 cc.)	7.8	5.9	1.88	1,625	.05	.60	2.83	2.22
XVIII	A.T.10 (3 cc.)	9.4	4.6	1.58	1,792	135	.61*	2.84*	2.40*

* Fecal collections of period XVIII were incomplete and values were calculated, using fecal nitrogen excretion as an index.

muscularly for periods X and XI. A.T.10 in a dosage of 3 cc. (3.75 mg.) per day, was started with period XII and was given throughout the remainder of the study except for period XIII, when it was inadvertently omitted. Parathyroid extract was given for three days, namely, the last day of period XVI, and the first two days of period XVII. The dosage was 200 units daily given intramuscularly. The balance study was terminated on July 3, 1957, after 18 periods.

At the termination of the balance study, A.T.10 was discontinued, and the patient received 100,000 units (2.5 mg.) of vitamin D_2 daily by mouth (Fig. 4). The supplementary calcium gluconate was continued, and patient was given an ordinary low-phosphorus diet. Because of the reversal of the urinary Sulkowitch reaction and the slight decrease in the serum calcium level, vitamin D was discontinued, and A.T.10 (3

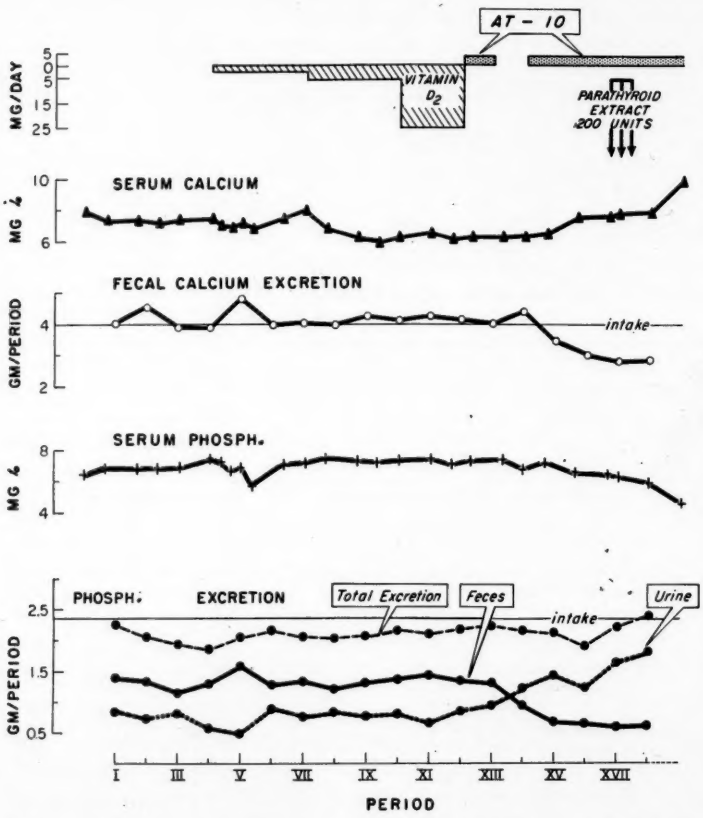

FIG. 2. Serum levels (mg. per 100 cc.) and excretions (Gm. per period) of calcium and phosphorus for the entire balance study. Each period was of three days' duration. During administration of vitamin D_2 there was no indication of correction of the hypoparathyroidism. The rise in fecal excretion of calcium and phosphorus at the beginning of vitamin D_2 therapy represents an intensification of the defect.

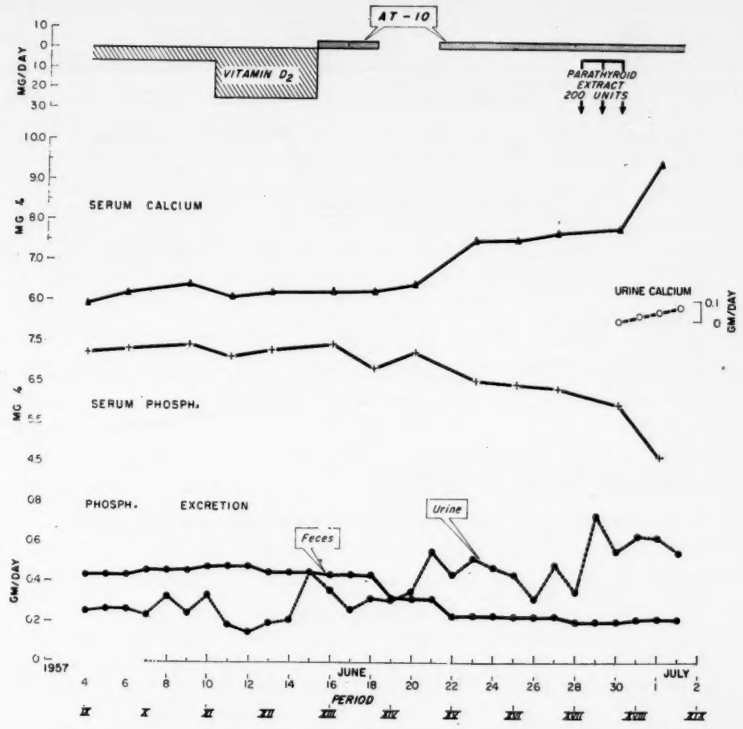

FIG. 3. Effect of A.T.10 in correcting the hypoparathyroidism that was unaffected by vitamin D_2. The return toward normal of serum calcium and phosphorus concentration (mg. per 100 cc.) and urinary phosphorus excretion (Gm. per day) occurred during A.T.10 therapy and before administration of parathyroid extract.

cc. daily) was started on July 18, 1957. The dosage was decreased to 1 cc. (1.25 mg.) daily, after seven days. The patient has been maintained on the 1-cc. dosage.

RESULTS

Results of the balance study are summarized in Table 1, and Figures 2 and 3. During the control periods (I, II and III) there was no significant net gain or loss of calcium or phosphorus. The abnormal serum concentrations remained stable. The major loss of both minerals was in the feces, which contained virtually all of the excreted calcium and 60 per cent of the excreted phosphorus.

Throughout the 8 periods in which vitamin D_2 was given, the serum calcium concentrations showed a distinct downward trend from the low control values, and serum phosphorus concentrations tended to be higher than during control periods. The only changes in calcium and phosphorus

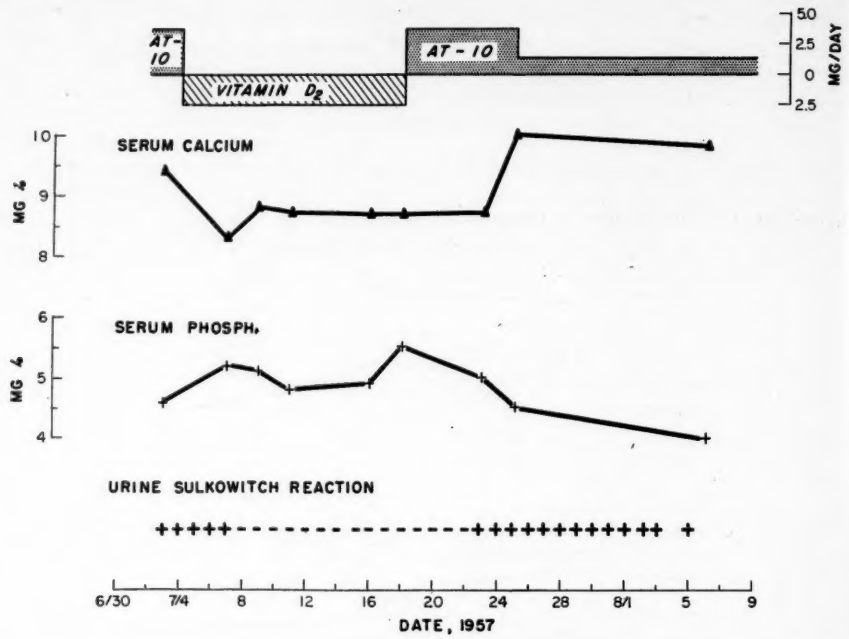

FIG. 4. Post-balance study. Vitamin D_2 did not maintain the normal serum values (mg. per 100 cc.) or the presence of calcium in the urine. A.T.10 restored the values to normal.

excretion during vitamin D_2 therapy were in the first two periods of administration, *i.e.*, periods IV and V. The urinary excretion of phosphorus decreased in periods IV and V. The fecal excretion of both calcium and phosphorus in period V was greater than in any other period of the study.

The effects of A.T.10 can be clearly seen in Figures 3 and 4. In Figure 4, which is plotted on a daily basis, the effect of A.T.10 is more distinguishable from that of vitamin D_2 or parathyroid extract. Administration of A.T.10 was started in period XII. Despite the lapse of therapy in period XIII, the urinary excretion of phosphorus began to rise in this period, and continued to rise in periods XIV and XV. The decrease in fecal phosphorus excretion was not apparent until period XIV. In the following periods, it fell to half of its previous value. In period XIV, the urinary excretion of phosphorus exceeded the fecal excretion for the first time, and this occurred before any demonstrable change in calcium excretion. The decrease in fecal calcium excretion did not start until period XV, and it is in this period that serum calcium concentration began to rise, and serum phosphorus concentration began to fall.

Parathyroid extract, given in addition to the A.T.10 on the last day of period XVI and the first two days of period XVII, produced no striking changes. The urinary phosphorus excretion of period XVI was actually less than that of the preceding period, and the rise in period XVII was exceeded in period XVIII when only A.T.10 was given. Calcium excretion in the feces continued to fall in periods XVII and XVIII, with little change in fecal phosphorus excretion. The positive calcium balance that started in period XV continued to the end of the study. Serum concentrations of calcium and phosphorus reached normal levels in period XVIII, and it was at this time that measurable quantities of calcium first appeared in the urine.

Serum concentrations of citric acid were low during the control periods and did not change throughout the study, except for one value of 2.62 mg. per 100 cc. in period XIII. The other values ranged from 1.25 to 1.88 mg. per 100 cc.

The clinical manifestations of hypoparathyroidism in this patient were of a chronic nature and no clear-cut changes occurred during the balance study. There were no episodes of tetany before, during, or after the study. There was only one convulsive seizure during hospitalization and this occurred before the onset of the balance study. The trophic defects of the fingernails were already being pushed forward by normal nail growth in the last control period (Fig. 1). By period XVI the proximal one-fourth of each nail was of normal structure; three weeks after the study was completed, the proximal half was normal; and two months after the study, the defect could be seen only at the distal tip of some of the fingernails. Toward the end of the study the patient displayed some paranoid tendencies. These became severe seven weeks after the study was over, and a psychiatric diagnosis of chronic schizophrenic reaction, paranoid type, was made.

At the end of the balance study the patient was given the hospital low-phosphorus diet. Supplementary calcium gluconate was continued. A.T.10 was stopped and vitamin D_2 in a dosage of 100,000 units (2.5 mg.) per day was administered orally. In Figure 4, serum calcium and inorganic phosphorus values and urinary Sulkowitch reactions are shown. Calcium was demonstrated in the urine for only four days after the change of medication. During the fourteen days of vitamin D_2 therapy, serum calcium concentration fell, and serum phosphorus concentration rose. A.T.10 was reinstituted in a dosage of 3 cc. (3.75 mg.) daily for five days, and then 1 cc. (1.25 mg.) daily. Calcium reappeared in the urine after five days of A.T.10 therapy, and serum values returned to normal. The patient was maintained on A.T.10 (1 cc. daily) without any regression of serum calcium or phosphorus to abnormal levels.

Right iridectomy was performed on July 30, 1957. Up to that time, no

changes had been noted in the right cataract in the interval since admission.

DISCUSSION

There are no specific clinical manifestations of hypoparathyroidism. The diagnosis should be considered in patients with tetany, convulsive seizures or lenticular cataracts. Since the diagnosis cannot be made unless the serum calcium level is low, repeated determinations at intervals may be necessary. In the present case, convulsive seizures brought the patient to medical attention. The clinical history suggested hypoparathyroidism, but the normal serum calcium concentration did not permit this diagnosis. Five years later, the presence of lenticular cataracts again suggested hypoparathyroidism, but the normal serum calcium and phosphorus levels precluded this diagnosis. Six years after the initial symptoms, when the patient entered the hospital for eye surgery, the serum calcium and phosphorus values were abnormal for the first time, and the diagnosis of chronic hypoparathyroidism could finally be established. No subsequent serum calcium or phosphorus value was normal until after therapy. At this time, six years after the initial symptoms, a sophisticated physician might well have made the diagnosis on the basis of epilepsy, cataracts, and trophic fingernail changes.

The signs and symptoms of chronic hypoparathyroidism fall into 2 general groups: 1) changes in the functional activity of muscular and neural tissues, and 2) structural changes of ectodermal tissue. In the first group are the tetany and gastro-intestinal symptoms, convulsive seizures, mental retardation, neuroses and psychoses. In the second group are the lenticular cataracts, and changes in the skin, nails, and hair. The papilledema does not fit well into either group.

The tetany and convulsions are usually the first to appear, as they did in this patient. These are directly attributed to the effect of the low calcium concentration on the irritability and conductivity of nerve and muscle tissue. It is of interest that these abnormalities were minimal at the time the patient was being studied. The basis for the cataracts is not known. They do not regress when the hypoparathyroidism is corrected, but further changes are halted. The changes in the fingernails have been attributed to moniliasis (10). In this case the complete healing of the fingernail defect in conjunction with the treatment of the hypoparathyroidism strongly suggests that moniliasis, when present, is a secondary complication. In this patient, there were no changes in the skin or hair. A recent report has directly linked a case of exfoliative dermatitis to hypoparathyroidism (11).

The renal handling of phosphorus by this patient was characteristic; almost all of the phosphorus filtered by the glomeruli was reabsorbed by the renal tubules. Evaluation of this function is not standard. One group

calculates the percentile reabsorption of filtered phosphorus (12–14), whereas other groups use the ratio of phosphate to creatinine clearance, and the serum phosphorus level (15, 16). Using either type of evaluation, the results in our case indicated hypoparathyroidism. The absence of skeletal defects, and the normal response to intravenous parathyroid extract excludes the sub-classification of pseudohypoparathyroidism (17, 18). The normal acid-base balance of the serum, and the absence of glycosuria minimize the possibility of a congenital renal metabolic defect. There was no evidence of intrinsic renal disease. Roentgenograms of bone did not show any changes except suggestive ones in the maxilla and mandible. The electrocardiogram did not indicate an increased rate of myocardial conductivity.

The use of a constant fixed diet throughout the balance study provided a stable intake of calcium and phosphorus, and a stable acid-base content. Variations of these factors have been shown to alter calcium and phosphorus metabolism and the response to therapy (19–21). During the 3 control periods of the study, the typical abnormalities of calcium and phosphorus metabolism in hypoparathyroidism were demonstrated, namely, a low serum calcium level and lack of calcium in the urine, and a high serum phosphorus level with more phosphorus excreted in the feces than in the urine.

The failure of vitamin D_2 to change any of the abnormalities towards normal is apparent (Fig. 2 and Table 1). The only changes that occurred during the 8 periods of administration of the vitamin enhanced the abnormalities. Early studies of the action of vitamin D demonstrated a diphasic effect, *i.e.*, an initial increased fecal loss of calcium and phosphorus followed by the therapeutically effective increased gastro-intestinal absorption of these elements (19–22). It would appear that in this study, the only effect of vitamin D_2 was the first phase, which increased the metabolic defect.

The effects of A.T.10 in this study (Figs. 2 and 3 and Table 1) were similar to those previously described (1, 2). First, urinary phosphorus excretion increased concomitant with decreased fecal excretion. Then fecal calcium excretion decreased at the same time that serum calcium and phosphorus values started toward normal. Calcium appeared in the urine only after the serum level was almost normal. All of the changes in calcium and phosphorus toward normal occurred during treatment with A.T.10 alone. Parathyroid extract given in conjunction with A.T.10 did not produce any great acceleration of this process. Urinary calcium excretion did not take place until after parathyroid extract was given.

Intramuscular administration of vitamin D_2 has been reported to be completely ineffective in correcting hypoparathyroidism (23). If this be correct, the results of this study are only quantitatively affected, since the

highest oral vitamin D_2 dosage was 250,000 units (6.25 mg.) per day as compared with the effective A.T.10 dosage of 3.75 mg. per day. The post-balance portion of the study shows that whereas 1 cc. (1.25 mg.) of A.T.10 maintained a remission, 100,000 units (2.5 mg.) of vitamin D_2 orally did not.

The interrelationships of the 3 pharmacologic agents used in the treatment of hypoparathyroidism have not been clarified. The sequence of events, as the metabolic defects are corrected, is different for each agent; moreover, the speed at which they work varies. Two factors separate parathyroid extract from the other agents: 1) the lack of response in pseudohypoparathyrodism, and 2) the development of ineffectiveness with continued use. The data in the case presented here, and the recent report of the development of unresponsiveness to vitamin D_2 in hypoparathyroid patients (24) seems to separate the action of vitamin D_2 from that of A.T.10. In the absence or diminution of normal parathyroid function, vitamin D_2 or A.T.10 can usually replace the parathyroid hormone.

The focus of investigative attention in hypoparathyroidism has been centered on the absorption, excretion, and serum concentrations of calcium and phosphorus. However, in the recent past, diseases of the parathyroid glands and of calcium and phosphorus metabolism have been related to a part of the tricarboxylic-acid cycle, namely, citric acid (25). Normal serum concentrations of citric acid range from 1.9 to 2.6 mg. per 100 cc. (26), and low values in hypoparathyroidism have been reported in 2 cases (27). In the present study, serum citric-acid levels were low, and did not rise when serum calcium and phosphorus values returned to normal. Only 1 serum citric-acid level of 2.62 mg. per 100 cc. was markedly different from the rest of the values, and this occurred at the beginning of the effective A.T.10 therapy. It was, however, a single value, and cannot be emphasized. The hypocitremia found in this case, and the lack of change with adequate therapy, suggest that the citric-acid level may be subnormal in hypoparathyroidism before serum calcium or phosphorus levels become abnormal.

Acknowledgments

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Letters to the Editor

EFFECTS OF 2-METHYL-DIHYDROTESTOSTERONE AND OF TESTOSTERONE ON HUMAN PITUITARY GONADOTROPIN*

To the Editor:

Testosterone will induce temporary regressive changes in metastatic lesions in certain patients with carcinoma of the breast, but the incidence of such regression is relatively low. In an attempt to enhance the anticancer activity of testosterone, various alterations in its structure have been made. One steroid resulting from such alterations is 2α-methyl-androstan-17β-ol-3-one (2-methyl-dihydrotestosterone). Huggins and associates (1) found this compound to be a potent inhibitor of the growth of experimental mammary tumors in rats.

Clinical results of a study recently completed at the Mayo Clinic on the effect of 2-methyl-dihydrotestosterone on the metastatic lesions of mammary carcinoma have been reported elsewhere (2). Herein are reported observations made during this study of the effect of 2-methyl-dihydrotestosterone and of testosterone on the urinary excretion of human pituitary gonadotropin (HPG).

MATERIAL AND METHODS

A group of 42 postmenopausal patients ranging in age from 36 to 79 years was selected for study. Most of these patients had undergone the menopause spontaneously; in a few, ovarian function had been eliminated by oophorectomy or roentgen radiation. Objective evidence of progressing metastatic malignant disease was present in all patients, none of whom had previously received any type of steroid therapy. The study was conducted on a double-blind basis, and patients received either testosterone or 2-methyl-dihydrotestosterone in accordance with a previously arranged schedule of randomization.

The steroids were given intramuscularly as their propionate esters at a dosage level of 100 mg. three times weekly. Assays for gonadotropin in the urine were made in duplicate, according to the method of Albert (3), prior to the start of steroid therapy and at each subsequent visit to the clinic. Although the interval between clinic visits varied, monthly examinations were performed when possible. A total of 276 assays for HPG were made. When the code of the study was broken, it was found that 16 of the 42 patients had received testosterone and 26 had received 2-methyl-dihydrotestosterone.

* Clinical aspects of this study were supported in part by grant CY-3664 from the National Institutes of Health, United States Public Health Service.

RESULTS

The mean values of duplicate assays are listed in Table 1. For each steroid, the patients are divided in 2 groups, namely, 1) those in whom objective regression of metastatic disease occurred during hormonal treatment (regression), and 2) those showing objective progression of metastatic disease during treatment (progression).

In all 42 patients, the control values for HPG were in the postmenopausal range (3). However, the variation in control values precluded any correlation between the level of HPG before treatment and the subsequent clinical response to hormonal therapy. The means for the control values in the 4 groups did not differ from one another and overlapped completely in a range indicated by two standard deviations.

Of the 16 patients who received testosterone propionate, 2 showed regression and 14 showed progression of the metastatic disease. The behavior of HPG during therapy with testosterone appears to be unrelated to the effect of the drug on the metastatic lesions, as suppression of HPG (defined as "O" values) occurred in both the small group of patients showing regression and the larger group in whom progression was noted. Since assays were performed irregularly at monthly intervals or longer, the precise time at which suppression occurred could not be determined, although a rough approximation of the mean time at which it took place could be made. This was found to be ten weeks after onset of therapy with testosterone. Another estimation of the time necessary for suppression of HPG by testosterone can be calculated by plotting cumulatively the occurrence of suppression in the 12 patients who showed this effect. In this group, suppression occurred in 50 per cent of patients within nine weeks and in 100 per cent within nineteen weeks of the time hormonal therapy was begun. Thus, it appears that suppression of HPG excretion by testosterone does not occur rapidly. Therefore, it is of interest that 4 of the 14 patients whose disease progressed during treatment (Cases 15, 21, 29 and 34) showed reduction in the excretion of HPG but not suppression. In these 4 patients, the excretion of HPG was 5, 47, 20 and 9 per cent of the control values respectively, at eight, seven, ten and six weeks respectively after initiation of therapy. Because of the variability among patients, it appears likely that suppression of HPG would have occurred had these 4 patients received testosterone for more than ten weeks. Because of these time relationships, the behavior of HPG in these 4 patients cannot be regarded necessarily as being different from that observed in the other 12 patients who received testosterone.

Of the 26 patients who received 2-methyl-dihydrotestosterone, 12 showed regression and 14 showed progression of the metastatic disease.

TABLE 1. EFFECTS OF TESTOSTERONE AND OF 2-METHYL-DIHYDROTESTOSTERONE ON HUMAN PITUITARY GONADOTROPIN (RAT UNITS PER 24 HOURS)

Testosterone propionate									
	Weeks of therapy								
Case No.	0	1–5	6–10	11–15	16–20	21–30	31–40	41–50	51–70
	Regression								
20	99		0						
22	124		8	0		0	0	0	0
Mean	112		4	0		0	0	0	0
	Progression								
9	141		8	0					
11	65	0							
13	81		0						
15	178		9						
21	57		27						
27	76		20	13	0	0		0	
29	75		15						
31	130	33	0	0					
32	320		170	0					
34	107		10						
36	48	52	0						
40	52	0							
42	79		6	0					
45	106			0					
Mean	108	21	24	3	0	0		0	

2-Methyl-dihydrotestosterone propionate									
	Weeks of therapy								
Case No.	0	1–5	6–10	11–15	16–20	21–30	31–40	41–50	51–70
	Regression								
4	101		114	178	93	231	204	150	86
5	63	101	161		79	146	57		
8	80		68	53		137		78	40
10	69		101	158		100	52		
14	112	119		58	107	243	66	10	
16	32		136	131		110	144	50	333
24	80		166			60	23	50	
26	97	210	150	96	141				
35	82	60		37		79			
39	158		152	98					
43	68		37	46					
44	62		82	267		68			
Mean	84	123	117	112	105	130	91	68	153
	Progression								
7	684	228							
12	30		0						
17	213		264						
18	47		36	51					
19	72		37						
23	114		111						
25	123		591						
28	54	33							
30	152	166							
33	102		88		63		27		
37	178		73	146					
38	48		46						
41	80		78						
46	66	255							
Mean	140	170	81	99	63		27		

TABLE 2. SUMMARY OF THE EFFECTS OF TWO ANDROGENS ON HUMAN PITUITARY GONADOTROPIN

Drug received and clinical result	No. of patients	Effect on HPG	
		Suppression	No suppression
Testosterone propionate			
Regression of metastatic lesions	2	2	0
Progression of metastatic lesions	14	10	4 (?)
2-Methyl-dihydrotestosterone propionate			
Regression of metastatic lesions	12	0	12
Progression of metastatic lesions	14	1	13

Values for HPG in those experiencing remission were not altered, the mean of the assays at various time intervals remaining roughly constant. In the 14 patients whose disease progressed during treatment, suppression of HPG occurred in 1 instance; in the other 13 patients in this category, values for HPG were unchanged.

A summary of the effects of these 2 androgens on HPG is presented in Table 2. The entry in the "no suppression" column of the group receiving testosterone is indicated as being questionable, since it appears likely that this entry would have been zero had subsequent assays been performed on these 4 patients.

COMMENT

Various analogues of testosterone have been tested previously for their inhibitory effect on metastatic carcinoma of the breast. Such studies have indicated a close correlation between the anticancer activity of a compound and its effect on urinary gonadotropin. Analogues of testosterone that do not exhibit a suppressive effect on gonadotropin have been found consistently to be ineffective as anticancer agents (4). Therefore, it has been generally assumed that the inhibitory effect of androgenic hormones on metastatic carcinoma of the breast is associated with a suppressive effect on the gonadotropic function of the pituitary gland (5). However, results of the present study are at variance with such a concept and indicate that the anticancer activity of such hormones is independent of their effect on the gonadotropic activity of the pituitary gland.

Since the plan of the study precluded the testing of 2-methyl-dihydrotestosterone at various dosage levels, it cannot be stated whether this compound in doses larger than those used in this study has any suppressive action on HPG.

SUMMARY

Testosterone propionate and 2-methyl-dihydrotestosterone propionate were administered intramuscularly in dosages of 100 mg. three times weekly to patients with metastatic carcinoma of the breast. Testosterone suppressed excretion of gonadotropin, whereas 2-methyl-dihydrotestosterone did not. Regression of metastatic lesions was independent of the effects of these steroids on the excretion of gonadotropin. Suppression of the gonadotropic function of the pituitary gland is not, therefore, a prerequisite for the clinical success of androgenic hormones in the palliative treatment of patients with metastatic mammary carcinoma.

CHARLES M. BLACKBURN, M.D.
A. ALBERT, M.D.

Mayo Clinic and Mayo Foundation,
Rochester, Minnesota,
January 24, 1959

Addendum

Since the preparation of this communication, Segaloff and associates (6), reporting on the effect of 9α-fluoro-17α-methyl-Δ^4-androsten-3-one-11β,17β-diol (fluoxymesterone) in patients with metastatic carcinoma of the breast, noted failure of the hormone to suppress urinary gonadotropin to zero values in 8 of 14 patients.

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URINARY STEROIDS IN 10 CASES OF ADRENAL CARCINOMA, WITH SPECIAL REFERENCE TO PREGNANE-3α,17α,20α-TRIOL-11-ONE

To the Editor:

It has been pointed out in previous publications (1–3) that "pregnanetriolone" (pregnane-3α,17α,20α-triol-11-one) is excreted in the urine of cases of congenital adrenal hyperplasia (female pseudohermaphrodism and macrogenitosomia praecox) and also in a few cases of Cushing's syndrome, in some of which adrenal hyperplasia was verified by histologic examination (4, 5). By contrast, no pregnanetriolone was found in the urine of 254 normal adult males and females and 5 patients with adrenocortical tumor (6).

These findings led us to postulate the feasibility of a test based on pregnanetriolone excretion, for differential diagnosis between adrenal tumor and adrenal hyperplasia. However, since the number of cases of adrenal tumor studied with pregnanetriolone determinations was limited, we were careful to suggest that more such cases should be studied before any generalization could be made.

Recently we received through the courtesy of Dr. Roy Hertz of the Endocrinology Branch, National Cancer Institute, Bethesda, Maryland, samples of urine from 10 patients with adrenal carcinoma. We analyzed these urines for pregnanetriolone, pregnanetriol and hydrocortisone. The methods used are described elsewhere (2, 3).[1] Analyses for 17-ketosteroids (17-KS) and corticosteroids were performed at the National Cancer Institute, and results pertaining to 5 patients (V.G., G.T., V.F., R.S. and P.V.) together with a description of the cases have been published by Dr. Hertz and collaborators (7). Clinical data concerning the other 5 patients (G.H., J.B., L.S., T.R. and J.H.) and the respective excretions of 17-KS and corticosteroids were kindly communicated to us by Dr. M. B. Lipsett of the National Cancer Institute.

In Table 1 the urinary steroid data on the 10 patients with adrenal cancer are summarized. Mean excretions in normal adults and in patients with congenital adrenal hyperplasia (female pseudohermaphrodism and macrogenitosomia praecox) are listed for comparison. The following features seem to be noteworthy:

1. None of the adrenal cancer patients excreted pregnanetriolone in quantities detectable by our method, the sensitivity of which was in most cases between 0.1–0.2 mg. per twenty-four hours.[2]

[1] In all cases reported in the present communication, hydrolysis of urine was carried out with β-glucuronidase (Ketodase).

[2] In previous publications (1, 2) the sensitivity of the estimation was indicated as

TABLE 1. URINARY STEROIDS IN 10 CASES OF ADRENAL CARCINOMA

Patient, Sex & Age	Clinical observations relating to hormonal effects	Pregnanetriolone (mg./24 hrs.)	Pregnanetriol (mg./24 hrs.)	Hydrocortisone (mg./24 hrs.)	Corticoids (mg./24 hrs.)	17-KS (mg./24 hrs.)
G.H. (m.) 16 mo.	Phallus slightly enlarged; no other virilizing signs	<0.04	< 0.20	<0.02	0.5	100–160
V.G. (f.) 28 yrs.	Cushing's syndrome	<0.15	5.6	1.90	48.0	150
J.B. (m.) 36 yrs.	Cushing's syndrome+virilization	<0.10	4.5	1.04	30–50	100–150
G.T. (m.) 33 yrs.	Cushingoid appearance	<0.20	< 1.0	<0.1	11.5	11.3
V.F. (f.) 45 yrs.	Cushing's syndrome+hirsutism	<0.16	1.09	<0.08	18.0	34.0
L.S. (f.) 55 yrs.	Virilizing syndrome with hirsutism	<0.10	3.36	0.48*	5–10	40– 50
R.S. (m.) 30 yrs.	Slightly Cushingoid	<0.11	13.0	0.71*	47.0	55.0
P.V. (f.) 30 yrs.	Cushing's syndrome+virilization	<0.13	6.1	0.57	43.5	36.4
T.R. (m.) 59 yrs.	No clinical evidence of hormonal effects	<0.20	6.7	0.32	5–10	80–150
J.H. (f.) 18 yrs.	No clinical evidence of hormonal effects	<0.14	5.8	0.53*	3– 8	3– 10
Normal adults (mean excretion)		<0.05	0.1–1.5	≤0.03	5– 15	8– 20
Congenital adrenal hyperplasia (mean excretion)		1–5	5–20	<0.03	1–10	30– 60

* Hydrocortisone was not completely separated from additional substances showing either blue or pink fluorescence, and the values should not be regraded as quantitative.

2. Excretion of pregnanetriol was elevated above "normal" in most of the patients, and was normal in a few others. No correlation was found between the occurrence of virilization and the concentration of pregnanetriol in the urine. Neither could 17-KS excretion be correlated with the development of a virilization syndrome.

3. Hydrocortisone was excreted in increased quantities in some of the patients showing clinical signs of Cushing's disease, but the increment was also observed in patients without clinical signs of excessive adrenocortical activity. Conversely, excretion of hydrocortisone within the "normal" range was found in patients showing some of the characteristic signs and symptoms of Cushing's disease.

The most prominent feature of the foregoing results is that in none of the 10 cases of adrenocortical carcinoma was pregnanetriolone excreted in the urine in quantities detectable by our method. This applied to all categories, regardless of whether the patients exhibited virilization, signs of Cushing's disease, or no clinical evidence of hormonal effects. The claim is not being

0.04–0.05 mg. per twenty-four hours. In the present series, due to scarcity of material, smaller aliquots of the 24-hour urine pool had to be used, thus reducing the sensitivity. Similarly, the sensitivity of the pregnanetriol and hydrocortisone estimation was proportionately decreased.

made that all patients with adrenocortical cancer do not excrete increased quantities of pregnanetriolone. Nevertheless, the uniform results obtained in the present series would suggest that elevated excretion of pregnanetriolone in such cases might be regarded as exceptional.

Similarly, in another series of 5 cases of adrenocortical adenoma (1 with Cushing's syndrome, 3 with virilization, and 1 without significant hormonal effects), pregnanetriolone was not found in the urine in amounts above 50 μg. per twenty-four hours. In 1 of these cases the sensitivity of the analytical method was increased to the point of detecting 1–2 μg. of pregnanetriolone in a 24-hour urine specimen, but no pregnanetriolone was found (6).

Regarding the excretion of pregnanetriolone in cases of congenital adrenal hyperplasia, the present evidence based on analyses performed by us in 26 cases (1, 2, 3, 6) and confirmed by others (8–11) is that in all patients with this condition appreciable quantities of pregnanetriolone are excreted in the urine. To the best of our knowledge not a single case of congenital adrenal hyperplasia has been reported in which the urine was analyzed for pregnanetriolone but none was found. Thus is seems that high excretion of pregnanetriolone in congenital adrenal hyperplasia is more or less a general phenomenon, although this would not exclude the possibility of exceptions.

Comparison of the data on pregnanetriolone excretion in patients with adrenal carcinoma on the one hand and congenital adrenal hyperplasia on the other, suggests that the latter condition may be distinguished by the occurrence of large amounts (usually milligram quantities in adult patients) of pregnanetriolone in the urine, despite possible exceptions. One such case was observed by us, in which pregnanetriolone was found in detectable amounts in the urine of a woman with a virilizing syndrome not due to a tumor. In 10 other patients with similar clinical manifestations no pregnanetriolone was detectable (6). The single case in which pregnanetriolone was excreted may be regarded as an intermediary, not fully developed form of female pseudohermaphrodism.

The distinction between Cushing's syndrome due to adrenal carcinoma and that due to adrenal hyperplasia appears to be more difficult. In 6 patients with Cushing's syndrome, in 4 of whom adrenal hyperplasia was confirmed and in 2 of whom the presence of a tumor was excluded, pregnanetriolone was found in the urine in quantities ranging from 50 μg. to 400 μg. per twenty-four hours (6). Since in the cases of adrenal cancer producing Cushing's syndrome described in the present publication, the urines were analyzed for pregnanetriolone at a comparatively low level of sensitivity (100–200 μg. per twenty-four hours), it cannot be stated conclusively that pregnanetriolone was not present in the lower concentrations found in

cases of Cushing's syndrome due to adrenal hyperplasia. Clarification of this problem may possibly be achieved by using a larger volume of urine for the analysis and by the introduction of a modification of the method for estimation of pregnanetriolone which allows for the quantitation of 2 μg. of the compound in a 24-hour urine specimen (6).

It follows that when the clinical status warrants a differential diagnosis between adrenal hyperplasia and adrenal tumor in a patient excreting urinary pregnanetriolone in detectable quantities, adrenal hyperplasia should be considered. Alternatively, if pregnanetriolone is not being excreted in detectable amounts, an adrenal tumor should be suspected. In this instance, high excretion of either 17-KS, pregnanetriol, or corticoids may be suggestive but not diagnostic of an adrenal tumor. On the other hand, the excretion of any of these compounds within normal limits should not be taken as evidence against the presence of adrenal neoplasia (*cf.* Patient G.T). The proposed test does not allow for the distinction between "idiopathic" virilism and a tumor of the adrenal cortex, since in both conditions the excretion of steroids as measured by our method appears to be nonspecific.

MICHAEL FINKELSTEIN, PH.D.
JUDITH SHOENBERGER, M. SC.

Hormone Research Laboratory,
Hebrew University-Hadassah Medical School,
Jerusalem, Israel,
November 20, 1958

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We are greatly obliged to Drs. Roy Hertz and M. B. Lipsett of the National Cancer Institute, Bethesda, Maryland, for having made available to us the urine of the patients with adrenal cancer, together with their clinical histories and the data on 17-KS and corticoid excretion.

The pregnane 3α,17α,20α-triol-11-one used as reference was kindly donated by Ciba, Basel, through the courtesy of Drs. A. Wettstein, G. Anner and R. Neher. We also wish to express our gratitude to Dr. W. Klyne, Postgraduate Medical School, London, for the gift of pregnane-3α,17α,20α-triol.

The authors are much indebted to Prof. B. Zondek for his kind interest in the investigation.

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

(For program, see the April issue of the Journal)

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University

of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

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November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

FIRST INTERNATIONAL CONGRESS OF ENDOCRINOLOGY

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels. Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, the committee suggests that each of our members make voluntary contributions to this fund. Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PURIFIED FOLLICLE-STIMULATING HORMONE AVAILABLE

The Endocrinology Study Section, National Institutes of Health, announces that it is prepared to distribute, free of charge to qualified investigators, limited amounts of purified sheep pituitary follicle-stimulating hormone. The preparation has been packaged in two forms: 1) vials containing 5 mg. for use only as a Standard in assay procedures; 2) vials containing 25 mg., for experimental use. The preparation is a sterile, lyophilized powder, free or nearly free of other hormonal activities. Detailed specifications will be distributed with each sample.

Application for supplies, either of the Standard or of the experimental lots, should be made by letter to Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, National Institutes of Health, Bethesda 14, Maryland. The letter should state briefly an outline of the intended work and an estimate of the amount needed. Applications will be screened by a subcommittee and allotments will be made according to the estimates of merit of the intended work.

The preparation and distribution of FSH is supported by funds granted by several Institutes of the National Institutes of Health. The program is supervised by a subcommittee consisting of Drs. Robert W. Bates, Roy W. Hertz, R. T. Hill, Warren O. Nelson, and Alfred E. Wilhelmi, Chairman. The hormone preparation was made at Emory University under the immediate direction of Dr. Stanley Ellis.

INTERNATIONAL SOCIETY OF INTERNAL MEDICINE

The 6th International Congress of Internal Medicine will be held in Basel (Switzerland), *August 24–27, 1960*. This Congress will be organized in conjunction with the Swiss Society for Internal Medicine. For further details, please write to the Secretariat of the 6th International Congress for Internal Medicine, 13, Steinentorstr., Basel, Switzerland.

BOOK REGISTER

Atlas of Tumor Pathology. Section XII, Fascicle 40. Transplantable and Transmissible Tumors of Animals. By HAROLD L. STEWART, M.D., KATHARINE C. SNELL, M.D., LUCIA J. DUNHAM, M.D., and SAMUEL M. SCHLYEN, M.D., Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Published by the Armed Forces Institute of Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the Division of Medical Sciences of the National Academy of Sciences—National Research Council, Washington, D. C. In this important and well illustrated volume, the transplantable and transmissible tumors of animals are discussed as follows: Introduction, historical review and methods; tumors of the skin, of melanin-forming tissue, of subcutaneous tissues, of muscle, of bone, of hematopoietic tissues, of the lung, of the alimentary tract, of the kidney, of the reproductive organs, of the mammary gland, of the endocrine glands, of the blood vascular system, of neural tissue, and tumors of undetermined site of origin and undifferentiated histologic pattern; with supplementary classifications according to site of origin and histology and according to animal host. The various tumors of the endocrine and reproductive systems were included because there has been great emphasis recently upon the effects of hormones and the relation of the endocrine glands to cancer; 378 pages; 287 figures; 4 color plates; 1959. American Registry of Pathology, Armed Forces Institute of Pathology, Washington 25, D. C. Price $3.50.

Biosynthesis of Terpenes and Sterols. Ciba Foundation Symposium, edited by G. E. W. WOLSTENHOLME, M.A., B.Ch. and M. O'CONNOR, B.A. Thirty-seven members from seven countries discuss: biogenesis and transformations of squalene; discovery and elucidation of mevalonic acid; alterations of cholesterol biosynthesis in liver cell fractions from rats in various experimental conditions; the enzymic synthesis of branched-chain acids; the biosynthesis of β-hydroxy-β-methyl-glutaryl coenzyme A and its conversion to mevalonic acid; new aspects of acetate incorporation into isoprenoid precursors; the mechanism of a rearrangement occurring during biosynthesis of cholesterol; semi-, mono-, sesqui-, and triterpenes as cholesterol precursors; biosynthesis of squalene and of cholesterol from mevalonic acid; biosynthesis of yeast sterols and the preparation of ^{14}C-labelled vitamin D_2; bile acids, formation and metabolism; species comparison as an aid in the study of the process sterols→bile salts; some aspects of acid-catalysed cyclizations of terpenoid polyenes; biogenesis of terpenes in moulds and higher plants; the biosynthesis of terpenoid compounds in fungi; the biosynthesis of carotenoids by micro-organisms; and a comparison of the incorporation of labelled CO_2, acetate and mevalonate into carotenoids in a number of carotenogenic systems. As the Chairman remarked, "the results of this symposium will be quoted for many years to come and be regarded as a landmark in the progress of this subject"; 312 pages; 102 illustrations; 1959. Little, Brown and Company, Boston, Massachusetts. Price $8.75.

Gynecologic Endocrinology. By GARDNER M. RILEY, Ph.D., Associate Professor of Obstetrics and Gynecology, University of Michigan Medical School, with a Foreword by NORMAN F. MILLER, M.D., Professor and Chairman of the Department of Obstetrics and Gynecology, University of Michigan Medical School, Ann Arbor, Michigan. Section I on Endocrine Physiology covers the pituitary gland, the menstrual cycle, cyclic changes in the genital tract, hor-

mone production in pregnancy, and the physiology of the breast. Section II (Clinical) covers the endocrine status of the fetus and newborn, puberty, menstrual dysfunctions, abnormal sexual development, endocrine aspects of infertility, endocrine aspects of disorders of pregnancy, menopausal endocrinology, and endocrine syndromes. Section III covers diagnostic procedures, the steroid hormones of the female, and pharmaceutical endocrine preparations. It is a concise guide-book for the treatment of female patients of any age; 330 pages; 72 figures (1 in color); 18 tables; 1959. Paul B. Hoeber, Inc., New York. Price $8.50.

La Cartographie Thyroïdienne. Méthode Cartographique et Applications Cliniques. By JEAN MARTIN, Ancien Interne des Hôpitaux, Assistant de Physique Médicale, presented as a Thesis (March 8, 1957) to obtain the degree of Docteur en Médecine, Université de Nancy, France (Prof. M. KELLERSHOHN, Physique Médicale). This thesis presents a number of points concerning the physiopathology of the thyroid, studied by means of radioiodine, particularly the morphology of the normal and of the pathologic gland. Part I (cartographic method) deals with radioactive iodine, measures for detection, counting, interpretation, calculation of error, experimental models, the contour and volume of the gland, practical points in examination, and establishment of standards. Part II deals with the cartographic appearance of the thyroid in various diseased states, and further data re interpretation and measurements; 314 pages; 112 figures; 1958. Soc. d'Impressions Typographiques, Nancy, France.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York, 36, N. Y.

Glaucoma. Third Conference, January 8–10, 1958, Princeton, N. J. Edited by FRANK W. NEWELL, M.D., Department of Surgery (Ophthalmology), The University of Chicago, Chicago, Illinois. The subject is discussed under the headings: tonography, osmotic factors in the formation of aqueous humor; consensual changes in intraocular pressure under experimental conditions; and tonography in the clinical management of glaucoma; 272 pages; 73 figures; 21 tables; 1959. Price $5.25.

Group Processes. Fourth Conference, October 13–16, 1957, Princeton, N. J. Edited by BERTRAM SCHAFFNER, M. D., University Seminar on Communications, Columbia University, New York, N. Y. The contents include: the impact of game-ingredients on children's play behavior; implications for our current models of personality; group size, interaction, and structural environment; and the role of aggression in group formation; 266 pages; 11 figures; 1959. Price $4.50.